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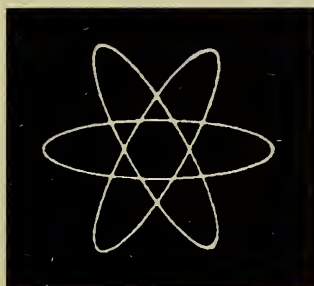
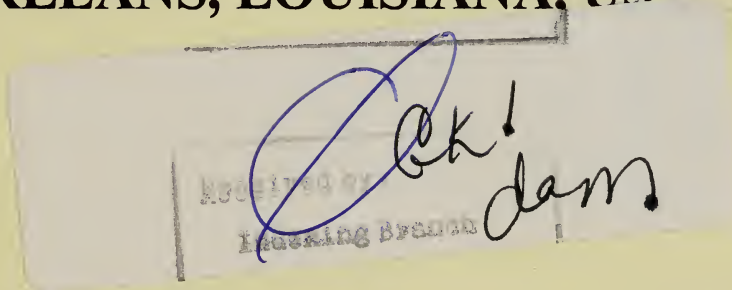
**PROCEEDINGS OF THE
1996 SUGAR PROCESSING
RESEARCH CONFERENCE**

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1996 SUGAR PROCESSING
RESEARCH CONFERENCE**

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**Sponsored by
Sugar Processing Research Institute, Inc.**

December, 1996

THE SUGAR PROCESSING RESEARCH INSTITUTE
OF THE
SUGAR BEET PROCESSING
INDUSTRY

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PREFACE

The 1996 Sugar Processing Research Conference is one of a series of Conferences held in alternate years to provide a forum for exchange of information among technical leaders of the sugar industry and to report on new and noteworthy developments. The Conference was sponsored by Sugar Processing Research Institute, Inc. (S.P.R.I.). The Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, contributed in kind to the organization of the Conference.

The program for this Conference was arranged by Margaret A. Clarke. The Conference Coordinator was Shirley T. Saucier. These Proceedings were edited by Margaret A. Clarke with Editorial Assistant Janell D. Becker.

The series, Proceedings of the Sugar Processing Research Conference, of which this is the eighth issue, continues the Proceedings of the Technical Sessions on Cane Sugar Refining Research, which was published every other year from 1964 to 1980. For individual copies of this volume as well as back issues of the former series as long as the supply lasts, write to Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Blvd., New Orleans, LA 70124. Before 1986, Proceedings were published by the Agricultural Research Service, U.S. Department of Agriculture. Since 1988, Proceedings have been published by the Sugar Processing Research Institute, Inc.

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1996 S.P.R.I. SCIENCE AWARD PRESENTATION

ENERGY ECONOMY OPTIMIZATION IN THE SEPARATION PROCESSES OF SUCROSE-WATER AND NON-SUGARS

Pascal A. Christodoulou, Hellenic Sugar Industry, Thessaloniki, Greece

ABSTRACT

The problem of energy economy in the separation processes of sucrose-water and sucrose non-sugars, which is of special importance with reference to the scarcity and high price of fuel, is approached by means of enthalpy and exergy (available energy) balances. The practical and theoretical limits of the energy demands of separation processes are examined.

Energy economy in the separation processes can be achieved in principle by limiting the consumption of vapours and electricity in a sugar factory. The function of an evaporation station is not only to evaporate water but also to distribute the resulting vapours to the different positions of consumption. By limiting consumption in the places where heat is needed, water elimination in the evaporation station becomes inefficient; more vapours should be conducted from the last stage to the condenser to achieve a high Brix in thick juice, thus increasing steam consumption. The storage of thick juice further decreases vapour withdrawal.

The application of sophisticated systems of high technology increases the electricity demand in the sugar factory. The ion exclusion process introduces new quantities of water in the system. To cope with all these problems, a model of a sugar factory is proposed using six or seven stages in the evaporation station, recompression of vapours, double use of vapours in the sugar house and high pressure and overheating of steam in the power station.

The application of combined power cycles, e.g. gas turbine-steam, is also examined. The use of falling film evaporators in all stages of the evaporation station is recommended. High heat transfer coefficients contribute to energy economy. Juice quality and juice processing technology play a significant role in heat and energy economy. The use of separation processes at low temperatures (cold liming, reverse osmosis, ion exchange, ion exclusion etc.) promotes the use of waste heat. The

principles of "pinch technology" can help us in the calculations of new installations or in the improvement of existing ones. In a comparison of two methods with the same technological advantages the one using less energy should be preferred.

INTRODUCTION

"The joy makes everything sparkling like precious stones and the gratitude is warm like the sun." So spoke the Greek author Ploutarchos 2000 years, ago. I am glad for the high distinction you honored me with and I am at the same time grateful. I hope with every modesty, to be allowed to ascribe a part of this honour to my colleagues, the personnel, of Hellenic Sugar Industry, with whom I have been cooperating since 1960, the year of H.S.I. establishment. I believe that a part of this honour I should give back to the esteemed members of S.P.R.I. Science Award judging committee who proposed my nomination Dr. Richard Riffer, Prof. Giorgio Mantovani and Prof. Frieder Lichtenhaler. I would like to express as well my appreciation to Dr. Margaret Clarke, Managing Director of S.P.R.I., to Mr. Nick Broughton President of S.P.R.I., and of the scientific committee of C.I.T.S., as well as to the other officers of S.P.R.I. for the excellent hospitality extended to me and for inviting me to come here in these magnificent surroundings of New Orleans and deliver this paper. I would like to dedicate this account to Professor Giorgio Mantovani, of the University of Ferrara, honorary President of the Scientific Committee C.I.T.S., former winner of this award and professor of some of H.S.I. scientific personnel, who followed his sugar technology course in Ferrara.

I have chosen as the subject of my presentation and as a basis for further discussion the topic: "Energy Economy Optimization in the Separation Processes of Sucrose-Water and Non Sugars". I thought it opportune on this occasion not only to report on technical matters but as well to refer to the Greek philosophers who may not have supplied the answers to the problems of science but at least have posed the questions. Their thoughts contain in seed many of the formulations of contemporary science. The Greek and Latin literature, furthermore, form the basis of our humanistic tradition.

About the word energy: To the basic forms of thought of Aristotle (384-322 B.C.) who together with Plato (427-374 B.C.) was one of the biggest and most influential philosophers of the West belongs the couple of conceptions he coined: FORCE (DYNAMIS) and ENERGY (ENERGIA). Energy means:

- Capacity to do things and get things done (force, vigour)
- Creation of values
- Energetically returned work, with the physical sense of energy and work definition of today.

It is worth noting that the word "energy" is encountered in the New Testament in the meaning of the godly act (Ephesians 1,19 3,7 3,20 Colossians 1,29 2,12) as well as the word "dynamis" which is often referred to there (Luke 24,49; Acts 1,8; 1 Peter 3,22; Revelation 7,12 and 11,17,). The word energy is also often used by the Thessalonian father of the Greek Orthodox church Saint Gregorius Palamas (1296-1359), stating that God expresses himself through His "energies"; namely, concrete benevolent acts. "Energy is eternal delight" wrote William Blake, (1757-1827) the English poet and engraver.

The second word of the title of this paper ECONOMY was coined by Xenophon, a Greek general, historian and author (430-354 B.C.), disciple of Socrates (470-399 B.C.), in his treatise ECONOMICOS, a discussion about the management of agriculture with complete operational data. The other words of the title OPTIMIZATION, SEPARATION AND PROCESSES are of Latin origin, although the etymological substratum of at least one word is Greek, "Separation" comes from the Latin word PAR, PARIS meaning pair, equal, similar, which derives again from the Greek proposition PARA, meaning "by". "Processes" comes from the Latin PROCESSUS, past participle of "procedere", to proceed, from the Greek proposition "pro" and the Latin "cedere", meaning to go.

The word SUCROSE is of Sanskrit origin, common in all modern languages, as well as the word water (HYDOR in Greek). Both are coming from onomatopoeia. Nevertheless the Greek word "glycys" meaning sweet is the origin of glucose, and fructose comes from the Latin "fructus". The soldiers of Alexander the Great (356-323 B.C.) brought back from their campaign to India a plant producing "honey without bees" and that was the cane. Cane sugar, considered as a valuable strengthening medicine, was produced in the islands Sicily, Rhodos and Cyprus in the medieval times. Ruins of a cane sugar mill are still existing in Cyprus.

SEPARATION PROCESSES

Speaking about separation processes it is interesting to define which are the separation processes applied in the sugar industry and what is the driving force of

separation. The separation of different components is made possible by density or weight, by size, filtering, molecular filtering, ultrafiltering, centrifugation, reverse osmosis, pressing, ion charge (electrodialysis, ion exchange, chromatography etc), change in state (evaporation, concentration, distilling, drying, freeze drying etc), and diffusion. In the unit operation technique we can distinguish between thermal and mechanical separation operations. Thermal separation operations are : the extraction, the rectification, the evaporation and the crystallization. Mechanical separation operations are: the crushing, the filtration, the sifting, the centrifugation and the sedimentation.(3) We meet all these separation operations in sugar technology, even rectification in the production of alcohol from molasses.

Usually when we speak of separation processes in sugar technology we mostly mean juice purification and filtration. In the classical juice purification we eliminate the non-sugars, not totally, but only about 30%. The remaining non-sugars are separated in the three steps of the crystallization process which has a higher efficiency than the proper juice purification of approximately 70%. The non-sugars, not removed in the classical juice purification, accompany the juices till the end and are found finally in the molasses. We name these non-sugars, not eliminated in the classical juice purification, melassigenic. But water is also melassigenic and has to be separated before crystallization, in the evaporation station. The scope of this presentation is to discuss the energy economy optimization in a sugar factory where separation processes take place. In reality the whole procedure of sugar extraction from beets or cane or from raw sugar is a series of separation processes. Now the question is posed as to why to separate the sugar from the non-sugars. If the clients want brown or soft sugar let them have it. In this way the energy cost for separation will be diminished (33).

ENTHALPY AND EXERGY BALANCES

To study and prepare proposals of energy economy ameliorations, it is necessary to calculate four balances:

- 1) A mass balance (Lavoisier 1743-1794)
- 2) An enthalpy balance (First law or axiom of Thermodynamics)
- 3) An exergy balance (Second law or axiom of Thermodynamics)
- 4) An economical balance to decide whether the proposed expenses and investments are justified from the economy point of view.

The first law of Thermodynamics was formulated by the German physician, J. R. Mayer, in the year 1842, while the second law of thermodynamics was expressed previously in the year 1824 by the French engineer and artillery officer, N.L.S. Carnot (1796-1832), who is considered as the father of Thermodynamics. Famous scientists contributed to the foundation of the science of Thermodynamics in the 19th century, like the English James Joule (1818-1889) who calculated the mechanical equivalent of heat; the German Rudolf J. E. Clausius (1822-1888) who introduced the notion of entropy, as measure of the irreversibility of one physical process (1865); the English William Thomson (Lord Kelvin) (1824-1907) who introduced the absolute (Kelvin) scale of thermometer; the American Josiah W. Gibbs (1820-1872) who introduced the notion of free energy and the English James Clerk Maxwell (1831-1879), law of velocity distribution of gas molecules (1860) and electromagnetic theory (1865). Progress was continued in the 20th century by the Germans Herman Nernst (1864-1941), Max Planck (1858-1947) and the French Jules Henry Poincaré (1854-1912).

Constantin Caratheodory (1873-1950) born to Greek parents in Berlin expressed the second law of thermodynamics without the notion of heat by introducing the principle of the adiabatic wall. Albert Einstein, when asked who he considered as his main teacher, named Constantin Caratheodory. In 1977 the Belgian I. Prigogin was awarded the Nobel prize for chemistry for his work on irreversible thermodynamics. I am dwelling on this to show that thermodynamics is a science branch, which is subject to constant development and is not an obsolete knowledge of the past.

It is worth noting how closely pure and applied research cooperate. A technical problem in the 19th century, that is, the recovery of work from heat in the steam machines, established one new region of physics, where engineers, physicians and physicists jointly interacted.

I would like here to state the opinion that the first and the second law of thermodynamics is the result of a long philosophical research, and in a seminal way have been expressed by the early Greek philosophers. Herakleitos of Ephesos called the dark, and also the philosopher of fire coined the principle of irreversibility saying : "You can not step twice into the same rivers, for fresh waters are ever flowing in upon you". By the word waters he means the conditions which never are the same but ever changing. He means also time.

Another famous passage of Herakleitos which is incorporating in seed the first and second law of Thermodynamics is: "This cosmos, which is the same for all, no one of

gods or man has made; but it was ever, is now, and ever shall be an ever-living Fire, with in measures kindling and in measures going out". If we substitute in the fragments of Herakleitos the word "fire" by the word "energy" we arrive at very interesting results.

Another passage which describes the four states of the matter: that is, solid, liquid, gas, and gases in fiery condition, corresponding to the words earth, water, air, fire, is the following: "Fire lives the death of air, and air lives the death of fire; water lives the death of earth, earth that of water" and the description of cold, warm, wet and dry conditions of matter: "Cold things become warm, and what is warm cools what is wet dries, and the parched is moistened". Another fragment of Herakleitos which can be interpreted in the way of thinking of a thermodynamicist who knows that without any difference of temperature, pressure, concentration, etc., there is no flow. "War (difference) is the father of all and the king of all; and some he has made gods and some men, some bond and some free". This reminds us of the free energy of Gibbs. The product of entropy and of absolute temperature is the bonded energy inside a system (anergy). The dices play is the basis of the theory of statistics: "Time is a child playing dices; the kingly power is a child's".

The principle of uncertainty and of the fuzzy logic: "We step and do not step into the same rivers, we are and are not "and" the wise is one only. It is unwilling, and willing to be called by the name of Zeus". And about separation which is our subject: "Even the posset separates if it is not stirred". The fact that separation of a precious component from others less desirable needs tremendous effort and energy is expressed by Herakleitos as well: "Those who seek for gold dig up much earth and find a little". Herakleitos of Ephesos in his axiomatic way of speaking prefigures the mathematical and physical sciences of 19th and 20th century, which gained exactness and clarity by the axiomatic methods.

Democritos (460-390 B.C.) expressed the principle of mass and energy conservation by these words: "Nothing comes into being out of nothing and passes away into nothing" or in Latin "Ex nihilo nil fit, nil fit ad nihilum".

At this point, it is interesting to underline that most of the words used in thermodynamics, as in other disciplines of science, are of Hellenic (Greek) origin as for example: Adiabatic, Air, Anergy, Axiom, Basis, Bar, Cybernetics, Cycle, Diagram, Dynamic, Economy, Enthalpy, Energy, Entropy, Exergy, Fire, Free (from the goddess of the German pantheon Freia, corresponding to the goddess and planet Aphrodite or

in Latin Venus), Gas (from Chaos), Hygrometer, Ideal, Isobar, Isochor, Isotherm, Kilograms, Kinetic, Liter, Logarithm, Manometer, Mass, Mechanism, Parameter, Phenomenon, Piezometer, Phase, Pycrometer, Polytrope, Rheologie, System, Statistic, State, Thermodynamics, Thermal, Technique, Thermometer, Xeros, Work, Zone.

Some of the above words descend directly from the ancient Greek literature, like energy and economy, as we have discussed previously and some were coined by contemporary scientists who used Greek "lemmata": like "entropy" coined by Clausius which means that part of the energy which turns inside the system and is not any more available.

Or like the notion of exergy which was introduced in 1952 by Rant from the Greek word "ex" and "ergon" (work) meaning that part of energy which is available to do work. The exergy is determined by the conditions of the surroundings:

$$E = H - H_o - T_o (S - S_o)$$

where E = Exergy, H = Enthalpy, T_o = absolute temperature of the surroundings, S = Entropy, H_o = Enthalpy of the surroundings, S_o = Entropy of the surroundings.

Using both the first and the second law of thermodynamics we can find the sources of losses and formulate proposals of improving our processes. Usually and unfortunately only balances based on the first law (enthalpy balances) are applied. Use of enthalpy balances in the sugar industry determines the quantities of steam necessary for heating, evaporation or crystallization; temperatures are calculated during cooling or condensation of vapours, as well as the heating surfaces of apparatuses and the coefficients of heat transfer.

The exergy balances give us a complete figure of the energy economy as well as of the thermodynamic efficiency of the processes and the installations; they unveil the thermodynamic losses resulting from the entropy increase or the exergy decrease, the losses during burning or heat transfer, during throttling, expansion, steam cooling, and the dissolving of sugar. All these losses are not indicated in the enthalpy balances. The advantage of an exergy balance is that the losses found can be converted directly to kwh which could be gained if those losses could be avoided.

By the exergy balances we can compare thermotechnical processes which operate or are conducted using other forms of energy (e.g. electrical or mechanical energy). There is also the possibility to compare the incoming and outgoing exergy flows.

The coefficient of exergy efficiency which is computed by the ratio of the exergy obtained to the exergy expended, characterizes the thermodynamic transformations, better than the enthalpy efficiency coefficient. As an example we can take the enthalpy efficiency of a steam boiler, with values of 92% independent of the steam overheating and pressure, while by increasing temperature and pressure we increase the exergy of the steam. While the enthalpy coefficient can take values up to 92% the exergy coefficients are much lower in the case of the steam boiler and can take values from 25 to 50% by increasing the steam pressure from 10 bar 200°C up to 300 bar 550°C. (13) (Figure 1)

In this way the exergy balances better evaluate the different processes and can locate the points where the real losses occur and enable us to take measures for their suppression or elimination. We know that the energy is not lost but degraded to other forms which have not the same capacity to do work. The energy as heat in the temperature and pressure of the surroundings is totally converted to anergy. The exergy losses in a heat exchanger are proportional to the square of the temperature difference. More information about the notions exergy, anergy and their application in the sugar industry is given in the references. (1-8, 10-17, 31).

HISTORICAL REVIEW OF THE ENERGY ECONOMY IN THE SUGAR INDUSTRY.

In the sugar industry as early as 1692 Antony Smith indicated the possibility of using steam for evaporation instead of direct fire as was customary in the open pots, also called battery or "equipage". Progress was made possible through basic inventions like the spray - condensation of vapours (1710), the evaporation under reduced pressure (Howard 1813) and the pan with tubes (1828). The real inventor of the multiple effect evaporator in vacuum seems to be Norbert Rillieux of Louisiana who had conceived of the idea as far back as 1832, but only took his first two patents out in 1843 (double effect) and 1846 (triple effect with horizontal tubular heating surface).

Multiple evaporation can be considered as one of the world's greatest economic inventions and literally millions of tons of fuel are saved yearly through its adoption in the sugar and other industries. Evaporation bodies with vertical tubes were introduced by Robert in 1850. The system of diffusion introduced about 1870 entailed a great dilution of the beet sugar juice and was a real incentive for evaporation improvement (24).

H. Claassen, in several editions of his book "Die Zuckerfabrikation" (1930-1943), has referred to a beet sugar factory with a steam consumption of 64 kg as typical and claimed that no sugar factory should use more than 70 kg of steam per 100kg of beets (22). (All steam and energy consumption quantities relate to the processing of 100 kg of beets or cane). K. Schiebl (1939) describes a beet sugar factory with steam consumption of 59 kg and steam boilers of 20 atmospheres pressure and 375°. The production of electricity is something more than 2 kwh (28) T. Baloh (1956), in heating technology calculations of the evaporating station for a 3000t/d beet sugar factory, mentions a steam consumption of 47.1 kg (1). F. Schneider (1960) reports that, in modern sugar works, a total of 40-50 kg of steam is used. The distribution of the steam is as follows: boiling pans 20-25, juice heating 15-18, extraction and radiation loss 6-12 kg. The electrical energy consumption amounts to 3.0 - 3.6 kwh for raw sugar works.

The steam boiler pressure is 30-60 atmospheres, the higher pressure being employed where electricity is supplied to the public mains (29).

T. Baloh (1963, 1964 and 1966) described a beet sugar factory with a steam consumption of 35 kg. The steam distribution was as follows: boiling pans 13.5 kg, sugar drying 1.2 kg, extraction 1.9 kg, juice heating in juice purification 14.9 kg, thin juice heating 5.3 kg and clear juice heating 1.8 kg. The thick juice is concentrated to 75 Brix. The boiler has a steam pressure of 75 bar and a temperature of 500°C. The turbine develops 5.77 kwh, of which 3.0 kwh is supplied to the works and 2.77 kwh to the public mains (2).

Marignetti and Mantovani (1974) proposed the replacement or combining of steam turbines with gas turbines, obtaining more economic installation, lower operating costs, simple operation and flexibility (27). G. Vernois (1975) reports that, since 1945, the sugar factories in France and Belgium employ mechanical thermocompression to a great extent. Steam consumption in white sugar manufacture is in this way greatly reduced to 28-36 kg(29).

P. Christodoulou (1977) proposed a model of a sugar factory having 5 stages in the evaporation station and a mechanical vapour compressor. The electrical energy consumption is 2.6 to 3.0 kwh / 100 kg of beets. Boilers pressure is 75 bar at 500°C. Steam consumption is 24.7 kg steam and the fuel consumption of 1.82 kg/100 kg of beets. Another alternative solution is the application of 6 stages in the evaporation station (10). This proposal especially concerning the low steam consumption (Figure

2) of the model in sugar house of 11% was disputed by G. Cossairt, who asserted that 11 % steam consumption in the sugar house needs a tantalizing effort (26). Nevertheless Baloh accepted and promoted my proposals (7). The same was made by Urbaniec (31). T. Baloh (1983) described a sugar factory using gas-turbine in the pulp-driers, vapour compression in the sugar house and in the evaporation station and boilers of low pressure steam for heating purposes (6).

P. Christodoulou (1989) proposed a sugar factory scheme with seven stages in the evaporation station using overall falling film evaporators with 13.8% on beets steam consumption, vapour compression from the second to the Ib stage steam chamber and compression of the vapours of A continuous boiling. The steam boilers have a pressure of 100 bar at 530°C; the fuel used is gas. A consumption of 3.4 MJ/Kg sugar is obtained. The theoretical energy required to separate sucrose from the non-sugars of sound beets is computed to be 5.9 MJ/100 Kg beets (14), (Table 1) (Figure 3).

In the new factories erected in the last three years in East Germany (1993-1995), part of thick juice is stored. The steam boilers have a pressure of 85 to 100 bar at 530°C. Seven stages evaporation station with falling film evaporators are used. The steam consumption is 15% on beets. The pulp drying by steam is applied. The energy consumption is 4MJ/Kg sugar. Some factories use gas turbines to increase the efficiency of electricity production. (23, 25) (Figure 4).

In Figure 5 is shown the steam consumption decrease % on beets in a model sugar factory as proposed by Claassen (1930), Schiebl (1939), Baloh (1956 and 1964), Christodoulou (1977 and 1989) as compared to real steam consumption in H.S.I. factories (1962, 1974, 1995) and in new factories in East Germany as well.

In Figure 6 is shown the evolution in steam pressure in a model sugar factory from 20 bar of Schiebl (1939) to the 100 bar of Christodoulou (1989) as well as the corresponding practical applications in sugar factories of H.S.I. and abroad. By the increase of steam pressure and overheating, the exergy losses become less; the cost of electricity production in the factory diminishes, and the energy requirements decrease as well (13).

In this way theory and practical application interact and influence the results, but as the seed contains potentially the plant, theory proceeds to the practical application. After the successful practical application a new theory can appear which results in better practical application and so on. I would like to exemplify the same idea in

another way using notions from the computers technology. First comes the conception of the software; then follows the hardware, then you can improve further your software.

Coming back to our sugar industry, in Table 2, the results of Hellenic Sugar Industry 1995 sugar campaign are presented. In Table 3 a comparison is made of the results of several sugar industries of Europe. Data were taken from a recent paper of Urbaniec (32) and from other sources (34, 35). Figure 7 shows the evolution of fuel consumption in Hellenic Sugar Industry: % beets for steam and electricity production and % on dry pulp. Similar curves are found in the literature (31, 32, 34, 35). More information about Hellenic Sugar Industry activities are given in the references (11, 15, 17, 18, 19, 20, 21).

THE IMPORTANCE OF ELECTRICITY ECONOMY IN THE APPLICATION OF SEPARATION PROCESSES

It is very important to take economy measures not only for thermal energy, but for electrical energy as well in the application of separation processes. Every quantity of thermal energy, which the sugar factory gives at the surroundings as waste heat charges on one side the environment and increases on the other side the fuel consumption. But every quantity of electrical energy which is wasted increases the fuel consumption three to seven times as much as the corresponding quantity of thermal energy which is saved at low temperature. According to the first law, the kwh of heat and electricity are of the same value, but according to the second law of thermodynamics, the kwh of electricity are pure exergy that is pure gold, while the waste heat at 60°C has an exergetical value at 20°C temperature of the surroundings of 0.14% of the kwh of electricity, that is seven times less. The waste heat at 60°C is like devaluated money. If the temperature of the surroundings goes down to 0°C the exergy value of the waste heat at 60°C becomes higher because the Carnot coefficient increases to 0.22 %. This explains why two absolutely similar factories in different geographical locations have respectively different energy economies. The factory at the northern location exploits better the fuel than the factory in a tropical or subtropical location. This is valid if we accept that both factories have taken the same measures of thermal isolation and apply absolutely the same processes.

An important step towards the energy economy is the application of reverse osmosis in the juice purification (3). The use of separation processes at low temperature (cold liming, reverse osmosis, ion exchange, ion exclusion, evaporation at low temperature

etc) promotes the use of waste heat and results in less energy loss to the surroundings, decreasing in this way the thermal energy demands (15). It is important at the same time to avoid excessive electrical energy usage.

PINCH TECHNOLOGY AND ENERGY REDUCTION IN SEPARATION PROCESSES

Pinch technology in a clandestine way applies the second law of thermodynamics, using graphical representations. Two composite curves, one hot and the other cold by heat exchange, are plotted in a diagram of enthalpy and temperature. By approaching the two curves we find the pinch point. By the application of this method we compute the minimum heat requirements of the heat exchange under examination. This in reality is the finding of the reversible heat transfer energy demands under zero temperature difference.

Three years ago I met, at a congress in Thessaloniki, Professor B. Linnhoff of the University of Manchester who is the founder of Pinch Technology and I asked him why he does not use the exergy notion. He responded that this is a difficult notion to explain to people and he prefers the graphical way which is more representative. I applied this method of Pinch Technology to recalculate the network of the heat exchangers of one factory of H.S.I. with excellent results (17). Similar results are reported by British Sugar (16). What is important is to think again and to find the errors of the existing installations.

SUMMARY AND CONCLUSIONS

In the last fifty years a real evolution in energy economy of separation processes in the sugar industry has been observed. From the steam consumption of 70% on beets of Claassen (1930 - 1943), a level of 15% steam consumption including the pulp drier was reached in the 1995 campaign. This results in lower cost of production of our sugar and lower load on the environment. To achieve these good results it was necessary to invest large amounts of intellect, research and money. The optimum solution would be to have the same results with less money.

Unfortunately the exergy conservation needs sophisticated equipment. It is very easy to destroy exergy. It is very difficult to conserve it. This is the task of the sugar technologists (engineers, chemists, agronomists, automaticians, managers, etc.): to produce sucrose with less and less cost, that is, with less energy, with less labour, with

less environmental load. A certain degree of "sustainable evolution" has been reached. Cane sugar, which uses a part of its own bagasse fibre instead of mineral fuel, is in a better position. The sugar industry in this respect of energy and environmental protection can be considered a prototype for other branches of industry.

We are then naturally led to another word coined by Aristotle: "ENTELECHY" which is the condition of a thing whose essence is fully realized, actuality as distinguished from potentiality. The word comes from the Greek "entelekheia", complete reality: "enteles", complete, full; "en" - in + "telos", perfection end, + "ekhein" to have. I wish our sugar industry, dear colleagues, would reach the highest degree of ENTELECHY possible.

SYMBOLS OF FORMULAS (Figure 1)

e	kJ/kg	Exergy of Enthalpy
E	kJ/s	Exergy Flow
h	kJ/kg	Specific Enthalpy
Hu	kJ/kg	Low Heating Value
m	kg	Mass
m	kg/s	Mass Flow
p	bar	Pressure
Qv	kJ/s	Heat Losses Flow
s	kJ/kg K	Specific Entropy
T	K	Thermodynamic Temperature
Zu	1	Exergy Boiler Efficiency
n	1	Energy Boiler Efficiency

Indices.

0	Initial condition	L	Air
1, 2, 3,	Conditions 1, 2, 3	SW	Feeding Water
A	Flue gases	u	Surroundings
B	Fuel	v	Losses
D	Steam		

el	Electric
h	Heating
K	Boiler

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Table 1. Adding the steam consumption of various heat users in a steam saving sugar plant. The steam consumption is calculated as saturated steam at 1 atmosphere (standard steam 2263 KJ/kg or 540 Kcal/kg heat of evaporation). Comparison of two proposals: a) Christodoulou (1977), 5 stages, Robert evaporators (10); b) Christodoulou (1989), 7 stages, falling film evaporators (14).

A) Christodoulou (1977), 5 stages, Robert evaporators (10). B) Christodoulou (1989), 7 stages, Falling film evaporators (14)

	Std. steam, Kg	Heating means		Std. steam, kg	Heating means
1) Extraction	0.9	Vapours 3	1) Extraction	0.6	Vapours 4
2) Heating of limed juice from 25 to 70°C amount 138 kg	-	waste heat (boiling vapours and condensate)	2) Heating of limed juice from 25 to 70°C, amount 138 kg	-	Waste heat (boiling vapours and condensate)
3) Heating of limed juice from 70°C to 85°C 85-70=15+6 deg.C. radiation loss=21 deg.C. Amount 138 kg <u>21 x 138 x 3.98</u> 2263	5.1	Vapours 5 and 4	3) Heating of limed juice from 70° to 85°C	5.1	Vapours 7,6,5 and 4
4) Heating of thin juice from 85° to 130°C, amount 120 kg <u>45 x 120 x 4.06</u> 2263	9.7	Vapours 4,3,2,1 and direct steam	4) Heating of thin juice from 85° to 125°C, amount 110 kg <u>45 x 110 x 4.06</u> 2263	7.9	Vapours 4,3,2,1 and direct steam
5) Sugar drying and sirup heating	1.8	Vapours 2	5) Sugar drying and sirup heating	1.1	Vapours 4
6) Boiling station	<u>11.0</u> 28.5	Vapours 3	6) Boiling station	<u>3.2</u> 17.9	
7) Deduct for expansion of vapours	<u>- 3.8</u>		7) Deduct for expansion of vapours	<u>- 4.1</u>	
Total steam consumption	<u>24.7</u>		Total steam consumption	<u>13.8</u>	

Table 2. Production results of Hellenic Sugar Industry (H.S.I.) - Campaign 1995.

Factory	Beets processed, tons			Boiler fuel % beets		Draft % beets	Kwh/ 100 kg beet	Boiler fuel % sugar	pol of beets	Sugar losses % beets
	Total	Daily	Best 10 Days	Total	Best 10 Days					
Larissa	556,449	6,912	7,416	2.93	2.73	117.2	2.93	26.3	14.4	0.89
Platy	661,390	7,715	8,951	2.74	2.43	111.8	2.82	26.2	13.6	0.79
Serres	420,569	4,732	5,153	2.80	2.51	115.0	2.66	24.3	14.4	0.47
Xanthi	412,060	5,937	6,824	2.66	2.39	113.0	2.65	24.1	13.9	0.54
Orestias	511,128	5,961	6,533	2.59	2.51	112.5	2.53	21.5	14.7	0.49
H.S.I.	2,561,596	31,257	34,877	2.75	2.52	113.8	2.73	24.6	14.2	0.66

Table 3. Production results of large sugar-producing companies in Europe 1994 Campaign (32, 34, 35).

Name	Country	Processing capacity t/d	Sugar output 1000 t	Unit fuel consumption (kwh/tb) Sugar manuf. Total *		Unit power consumption kwh/tb
Pfeifer & Langen	Germany	38,244	459	194	267	25.02
Sudzucker	Germany	110,702	1,186	191	267	30.60
ZN Nord	Germany	54,417	605	216	293	28.76
Suiker Unie	Holland	54,030	604	248	305	
DDS	Denmark	37,694	447		198	
Agrana	Austria	30,027	392	215	316	26.19
Sockerbolaget	Sweden	27,706	339		220	
	Poland	193,300	1,272	465	510	26.80
H.S.I. (1995)	Greece	31,000	287	245-306		27.50
	France	418,600	4,020	236		

* Including pulp drying

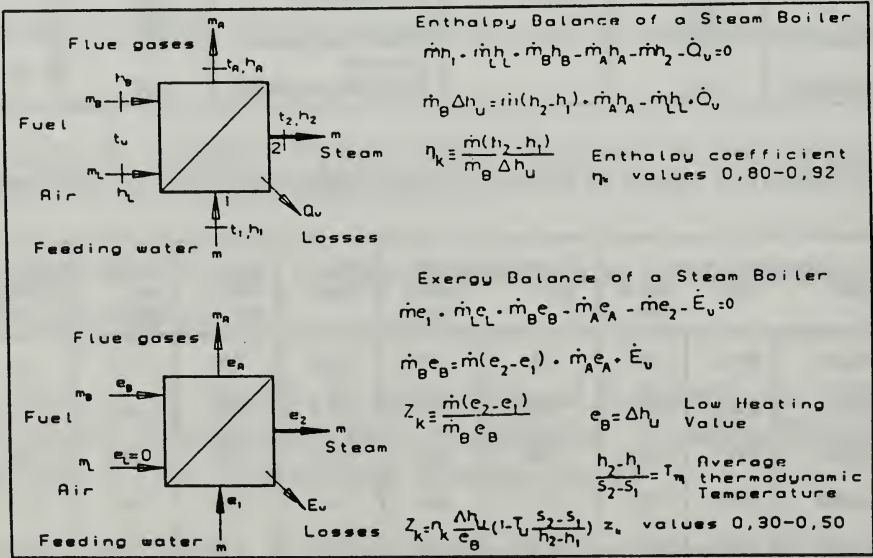


Figure 1. Enthalpy and exergy balance of a steam boiler.

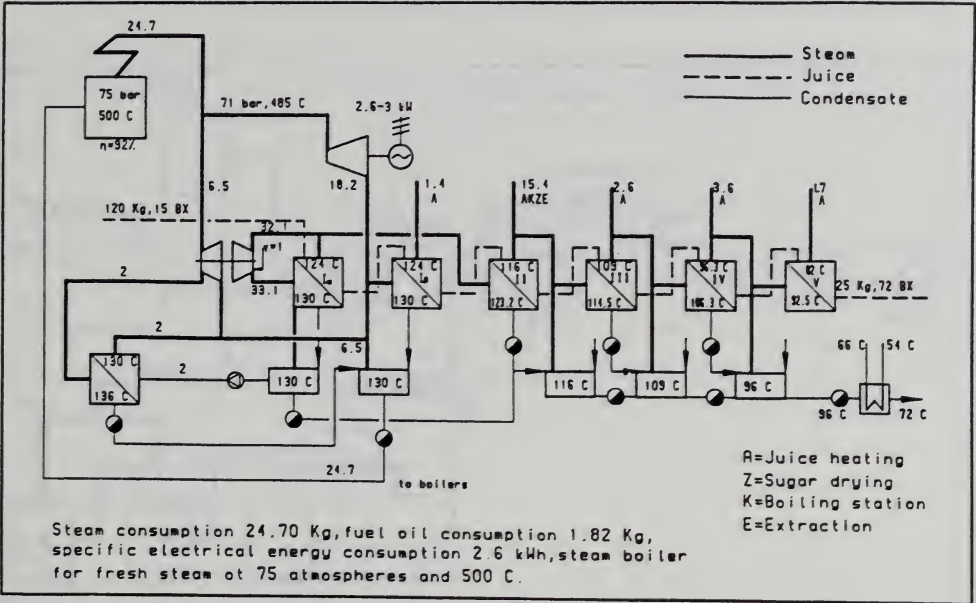


Figure 2. Scheme for a 5 stage evaporation station with vapour compression in the first stage, 1977 (10).

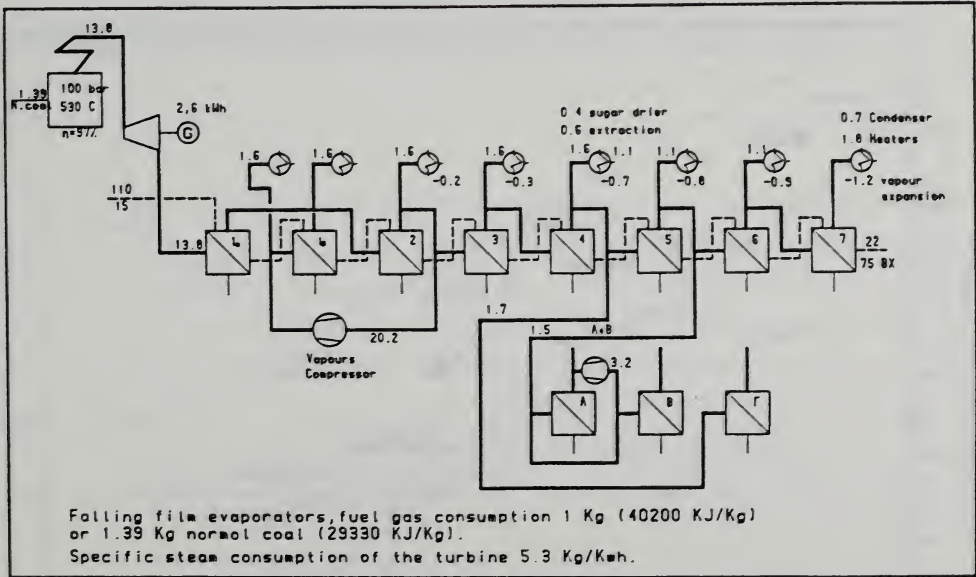


Figure 3. Scheme for a 7 stage evaporation station with vapour compression from the 2nd to the 1b stage steam chamber, compression of the vapours of A continuous crystallisation, 1989, (14).

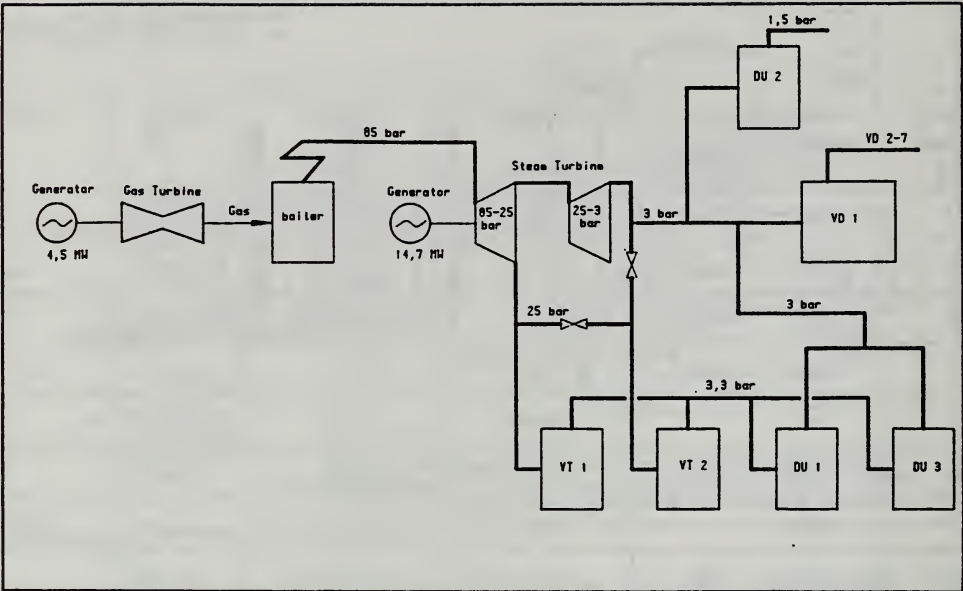


Figure 4. Steam system of Kleinwanzleben Sugar Factory (25). VT steam pulp drier
DU=steam transformer VD evaporation body.

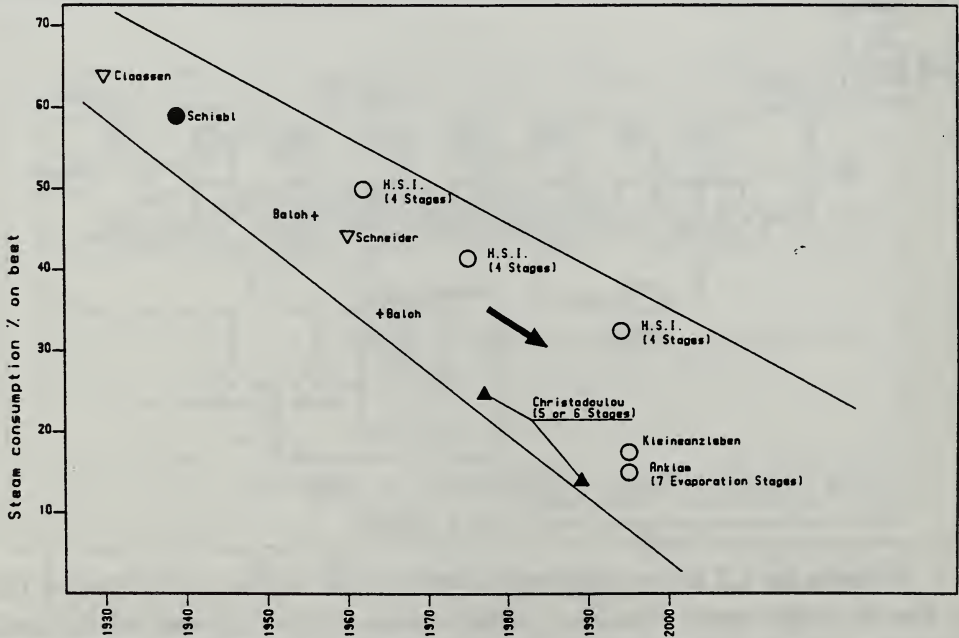


Figure 5. Steam consumption % beet in a model sugar factory.

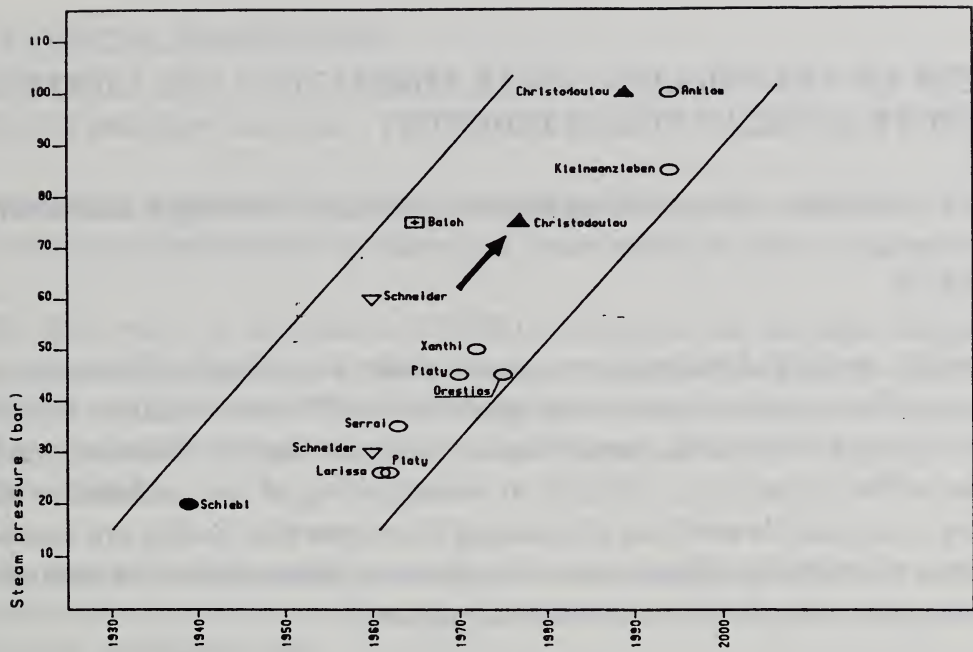


Figure 6. Steam pressure in a model sugar factory.

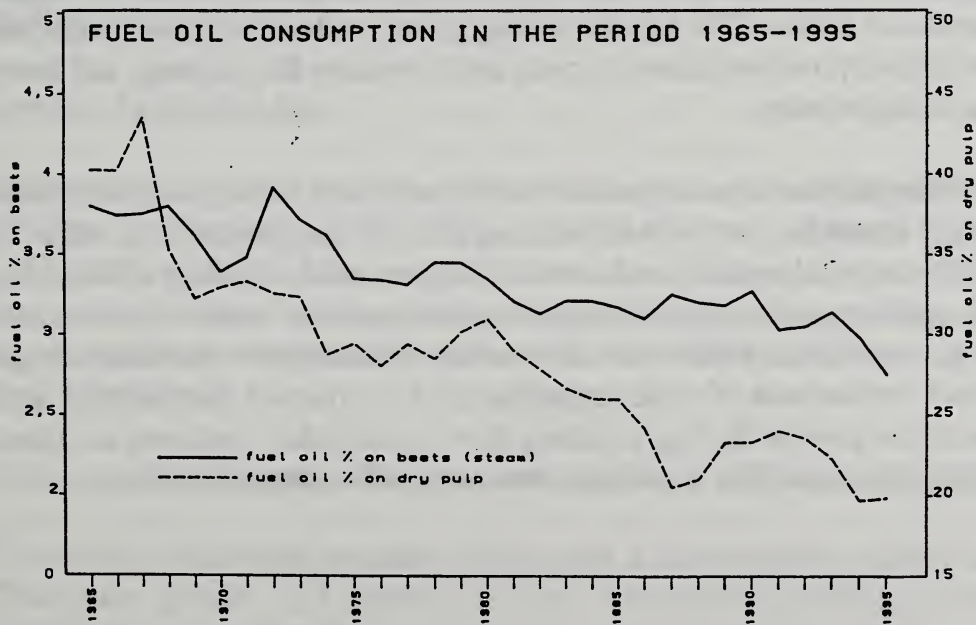


Figure 7. Fuel oil consumption in the period 1965-1995 in Hellenic Sugar Industry.

ASPECTS OF EVAPORATOR SCALE FORMATION AND CONTROL IN THE SOUTH AFRICAN SUGAR INDUSTRY

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ABSTRACT

A systematic method of analysing evaporator scales from South African sugar mills is described. The method is based on the use of XRD, XRF and HPLC to identify and quantify the major components present. Some results are given to illustrate trends and anomalies within the industry. Through an understanding of the mechanisms of scale formation some possible methods of reducing the evaporator fouling are considered. Procedures whereby the effectiveness of a particular treatment can be evaluated are discussed using examples from local investigations.

INTRODUCTION

A characteristic of evaporators in the cane sugar industry is their propensity for fouling resulting in a reduction in the rate of heat transfer and hence the capacity of the evaporator station. The benefits arising from a reduction in scale are therefore increased capacity, reduced cleaning costs and downtime for cleaning, and less sugar lost due to degradation.

Understanding of the scale composition and the processes leading to its formation are necessary to assess the methods of reducing or eliminating evaporator scale. To this end reliable analytical methods and a methodical process for dealing with the data are essential, and this paper outlines these procedures. Based on results obtained from the analysis of local scale, coupled with information obtained from the literature, general trends and mechanisms of scale formation can be proposed. Methods of assessing changes in evaporator fouling resulting from a particular treatment are illustrated using local examples. This work considers only mills using defecation.

ANALYTICAL PROCEDURE

Choice of analytical methods

The analytical procedure for scale should allow a reasonable estimate to be made of the main compounds present. In this regard three techniques were considered suitable:

- a. The first was x-ray fluorescence (XRF) which gives the chemical composition of the inorganic compounds, as well as a loss on ignition (LOI) at 1000°C. This technique can be combined with scanning electron microscopy (SEM) to provide semi-quantitative analysis of spots or small areas of a particular phase within a complex matrix.
- b. Ion exclusion high performance liquid chromatography (HPLC) was used to determine the composition of the organic fraction as organic acids using the method developed by Walford (26).
- c. X-ray diffraction (XRD) enables the crystalline phases present to be identified. Quantitative information can be obtained from the x-ray diffractograms although XRD patterns cannot be used as an absolute measure without standard samples being available for comparison.

Treatment of analytical data

While for many purposes a simple analysis of the basic components in a scale sample is sufficient, in other cases it is useful to establish the composition in terms of the compounds formed. The main compounds which may be present in scale, and the relevant ratios, discussed below, are summarised in Table 1. The procedure by which the quantity of each compound is estimated from the XRF, XRD and organic acid analysis is as follows:

- Silica can be considered to be present as a hydrated compound $\text{SiO}_2 \cdot \text{H}_2\text{O}$ (18).
- The oxalate (and other organic acid) content is calculated from HPLC analysis. The phase present (i.e. calcium oxalate monohydrate (COM) or dihydrate (COD)) is obtained by XRD.

- The total amount of phosphate is determined from XRF, and the crystalline phases formed can be established from XRD.
- Mg is reported as a hydrated compound ($\text{Mg}(\text{OH})_2$).
- The calcium mass balance is closed using $\text{Ca}(\text{OH})_2$ ("lime" or calcium oxide hydrate) which is estimated from a comparison of the amount of calcium analysed by XRF and that accounted for by the quantities of calcium phosphate and oxalate.
- The overall mass balance then gives the quantity of amorphous organic material in scale. Using the figures in Table 1, (that is LOI figures from the "ideal" case) an expected LOI can be calculated, using the estimated composition, and this can then be compared with the LOI obtained experimentally. Using an iterative process, in which the phosphate ratios are adjusted, it is possible to obtain an acceptable agreement between the calculated and the measured LOI. The process is summarized graphically in Figure 1.

RESULTS OF SCALE ANALYSIS

Analysis of evaporator scale has been the subject of many investigations worldwide over the years, and much of the work has been reviewed elsewhere (26). To obtain maximum benefit from the results of local scale compositions obtained as part of this investigation, these are considered in conjunction with work reported in the literature, and the following general observations regarding scale from Southern African mills can be made.

Trends across the evaporator train

The scale components can be broadly classified into three groups: phosphates, silica and silicates, and organic compounds, and in each case amorphous and crystalline phases are formed. (In the case of calcium silicate this is poorly crystallised). Phosphate and amorphous organic material decrease with increasing effect and silica and oxalate increase with increasing effect. Calcium is found throughout the evaporator train, being associated with phosphate in the first effects and with oxalate in the later effects. The organic compounds are not always well defined, either being amorphous material (no crystalline structure) or precipitated salts of organic acids. Some typical results obtained using the methods outlined are given in Table 2.

Phosphates

Phosphates are precipitated principally as calcium phosphate, either as amorphous calcium phosphate (ACP) only, or mixed with a crystalline phase. Since the formation of crystalline phosphates results in a more problematic scale than when ACP only is present, it is worthwhile considering some aspects of crystalline phosphate formation. While many crystalline phosphate phases have been identified (4, 9, 10, 20) the phases given in Table 1 were the only ones identified in the local scale samples analysed, that is, hydroxyapatite (HAP) $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$; collensite, or calcium phosphate hydrate (CPH) $\text{Ca}_2(\text{Mg,Fe})(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$; and brushite (BR) $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$. The precipitation of calcium phosphate begins in the clarifier where conditions of high supersaturation appear to favour the formation of ACP initially. Although the calcium phosphates have a very low equilibrium solubility, the precipitation is characterised by slow kinetics, which explains its continuation in the earlier effects. Several factors appear to affect the transition of ACP to crystalline solid, and the particular phase formed appears dependent on concentration of the precipitating species, pH, temperature, and the presence of other ions (4, 7, 20). Circumstantial evidence from local scale samples suggests that the HAP is formed as a result of the dehydration of ACP, with brushite and/or collensite as intermediates in some cases. This is illustrated in Table 2 which includes samples taken over several weeks from a local factory (Maidstone (MS)) and suggests that the transformation results from aging of ACP.

Amorphous organic compounds

Sugar and other organic material are trapped in the bulky phosphate precipitate and are degraded through the action of heat on the tube walls. Thus the fraction of amorphous organic material follows the same trend as the phosphate, i.e. it decreases as the evaporator effect increases. It was found that silica also appeared capable of trapping some organics in the scale but to a lesser extent. If the mechanism of formation is one of chemical reaction fouling (3), i.e. the organic material reacts with the wall surface, then the fact that temperatures are lower at higher effects suggests that less amorphous organic compounds will be deposited later in the train. Amorphous organic material does not represent a particularly severe fouling problem, indeed its presence makes the scale soft and easy to remove, whatever cleaning procedure is used. In fact the addition of phosphate midway in an evaporator train has been used to soften hard scales containing gypsum (19).

Organic acids

Of all the organic acids present in scale, the calcium salt of oxalic acid is traditionally the most troublesome from a fouling aspect. Calcium oxalate is found in two forms (14), weddellite ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$) (calcium oxalate dihydrate (COD)), and whewellite ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$) (calcium oxalate monohydrate (COM)) which is the thermodynamically stable compound at higher temperatures. The fact that oxalates are found mainly in the later effects of the evaporators has led to suggestions that formation may be the result of a solubility effect. However work at the SMRI has shown that the solubility of calcium oxalate in sugar solutions is negligible (26). Further work at the SMRI has indicated that oxalate is formed in the evaporators, rather than being carried in from the juice extraction stage, and that the precipitation is sufficiently rapid to ensure that any calcium oxalate formed will precipitate in the same vessel. It has been proposed that oxalate is formed from aconitic acid under oxidising conditions (26).

An interestingly different but exceptionally hard and difficult to remove scale, formed in great quantity at a midland factory (Noodsburg (NB)) during the 1994 season, was calcium magnesium aconitate (CAC). This particular scale is not widely recorded locally or internationally, and the diffractogram obtained did not appear in the standards of the official Joint committee on Powder Diffraction Standards (JCPDS) index. A sample of calcium magnesium aconitate was made and a diffractogram obtained to confirm the presence of the CAC phase. Interestingly, CAC has not been found at NB during the 1995 season, and has only been identified in small amounts at other mills. Since aconitic acid is commonly found as a major component of the organic nonsugar fraction and calcium is abundant this was a puzzling scale to understand in terms of a precipitation pathway. At this stage it can only be assumed that the drought conditions prevailing during the 1994 season resulted in conditions arising in the juice which favoured the formation and precipitation of CAC, which becomes increasingly insoluble at high temperatures and with increasing sugar concentration (17). However analysis of monthly juice composites did not reveal higher magnesium levels compared with other mills, and work is continuing in this area (11).

Silica

Silica is usually precipitated in the later effects (and in some cases in the pans) as amorphous silica or poorly crystalline calcium silicate. It is important that the presence or absence of quartz (QTZ) be established since it represents sample

contamination. Clearly the implications of a high silica concentration where the silica is present as amorphous silica is quite different to that when sand is present. The factors influencing the precipitation of silica during the evaporation of sugar juice are not well established. It is generally believed that the precipitation follows polymerisation and coagulation and that these processes are promoted at high temperature (18). It is likely that the precipitation pathway leading to silica deposition is a solubility change effect and this is shown graphically in Figure 2, which gives the solubility profile for a typical evaporator train, plotted using silica solubility data at different temperatures (27). Assuming a clear juice feed with a typical silica concentration of 50 ppm (on juice) the point at which silica is deposited (about 50 Brix) can be seen. Analysis of syrup samples from a number of mills suggests that the solubility data is correct and that the situation shown in Figure 2 is a reasonable model.

Silica can be introduced to the juice through amorphous silica contained in the fibre of the plant, which is taken into solution during the extraction process, through use of poor quality imbibition water, through the re-use of sweet water which has been passed through a diatomaceous earth filter, or through the dissolution of clay or silt carried in with the cane. The dissolution of quartz sand is not expected to be significant in view of the poor solubility displayed by quartz in water (18).

Microscopic examination

To investigate the way in which the scale components are arranged, optical and SEM examinations were undertaken. Spot and area analyses of the amorphous compounds established that certain components occurred together, namely silica and calcium; as well as phosphate, calcium and amorphous organic material. Silica was also found to have been precipitated alone with little occluded impurities. These observations suggest that the calcium silicate, silica, and calcium phosphate are formed as colloidal particles in suspension before being deposited around the crystalline material (oxalate). This suggests that suspended solids are as likely to deposit in the evaporators as are dissolved solids. It was also found that the scale is formed in distinct layers, each rich in a particular phase. Examination of thin sections established that the organic material was strongly concentrated in certain layers, and where COM and COD were both present these too were formed in layers. This suggests that during the evaporation process conditions occur periodically which favour a particular compound's deposition, such as "dryout", "pulses" of juice containing high concentration of contaminants, surges in steam pressure, flowrates, or pH changes.

The concentration of certain components in layers implies that where chemical cleaning, using caustic, is used it will attack the organic and silica rich layers, but may not readily penetrate the oxalate rich layers, preventing efficient attack and cleaning. In such cases alkali/acid cleaning may be more effective.

POSSIBLE METHODS OF REDUCING THE EFFECTS OF SCALE

Understanding of the fouling process allows some consideration of possible methods of scale prevention. Some approaches are as follows:

- Removal of chemicals which cause fouling through chemical precipitation, ion exchange or filtration, during, after, or as a replacement for clarification. From results reported removal of the "common denominator", calcium, alone will significantly reduce scale and this has been demonstrated by Thompson (25). The fact that oxalate is formed, with very low solubility, and rapid kinetics suggests that the removal of calcium to quite low levels would be necessary to eliminate oxalate scale completely by this method, and this has been found in the paper industry, in work designed to eliminate oxalate scale (5). While all the techniques of softening or demineralisation use well established technology, the major barrier to implementation of these techniques to date is the high capital and operating costs. However, a holistic approach which includes sugar recovery from molasses, as well as recent reported success with membrane filtration technology could make demineralisation or softening more likely to be used in the near future.
- Changes in operating conditions can take an extreme approach through the introduction of new evaporator design, or less dramatically, simply by changing the operating conditions of existing evaporators. The latter option is a particularly attractive one since it is considerably less costly than the former. Changes in temperature, flowrates and feed distribution can contribute to reduced rates of fouling (3), and the effect of feedrate is considered below.
- Chemical additions (antiscalents) are a well established approach, being crystal habit modifiers, complexing agents, and/or dispersants. From the discussion above, crystal habit modifiers or complexing agents could be possible methods of dealing with oxalate or crystalline phosphate, but for silica a complexing agent or dispersant would be required to prevent deposition. Antiscalent trials have produced results ranging from apparent success to increased fouling rates (1,6,9,10,13,14,16). The effect of chemical addition locally is considered below.

- Physical methods based on a simple physical cleaning effect, such as spiral turbulence promoters (static or dynamic), including “self cleaning” evaporators (3). Other, more controversial, methods with varied levels of success (or notable lack of success) reported are ultrasonic techniques, and magnetic and electromagnetic devices (2,8,15,21). Tests of a magnetic device are reported below.

MONITORING OF TESTS TO ASSESS METHODS OF SCALE REDUCTION

The effectiveness of any attempt at the reduction of fouling must be assessed in terms of two requirements; firstly the improvement of the rate of heat transfer, and secondly, a softer (or more easily removed) scale. Measurement of changes in scale composition is most easily carried out using analytical techniques outlined above, and can provide useful information regarding susceptibility to chemical cleaning, scale hardness, and effects of a particular treatment on a particular component of scale. Evaluation of changes in the rate of heat transfer can be quite difficult in practice (24). The overall heat transfer coefficient (HTC) (U) measured after time t (U_t) is related to the fouling resistance (R_f) and the HTC when the evaporator surface is clean (U_0) by the following relationship:

$$1/U_t = 1/U_0 + R_f$$

U can be measured using the modified Fourier equation

$$U = \frac{Q}{A \times dT}$$

Where A is the surface area, Q is the rate of heat energy transfer and dT is the temperature difference across the heat transfer surface. Since the fouling resistance is related to the thermal conductivity of scale (k) and the scale thickness (x) by:

$$R_f = x/k,$$

the fouling resistance gives a direct indication of the amount of scale deposited on the evaporator surface. The simple fouling model of Kern and Seaton (3) gives the fouling resistance (R_f) after time (t) by the following equation:

$$R_f = R_\infty - R_\infty \exp(-\beta t^2)$$

where R_{∞} is the asymptotic value of the fouling resistance and β is a constant. This method is particularly attractive since it produces data essential for the design and modelling of evaporator stations in a usable form. Where an entire evaporator train is to be examined and /or delta T measurements are unreliable or unavailable, as a compromise, the global measurement of heat transferred per unit area (Q/A) for a set of evaporators can be calculated, which will give an indication of the amount of scale formed.

Direct measurement of the mass of scale, although reliable, is difficult in the mill situation and some further investigation is necessary to obtain sufficient information to use the results for estimating fouling resistances. Measurement of the time taken to clean evaporators, time of operation between cleans, and visual inspections are all valuable indicators of changed performance but can be unreliable due to the subjective nature of the observation.

Use of fouling resistance to evaluate the effect of flowrate and evaporator type on fouling

A pilot evaporator consisting of three, full length (7 m) evaporator tubes, was run at different feedrates to determine the change in the HTC's (and hence the fouling resistance) over time. A detailed description of the pilot plant is given elsewhere (29). In all cases the feed juice used was sourced from the outlet of the main plant first effect evaporators (i.e. feed to the second effect). The results of these tests are shown graphically in Figure 3 which shows the magnitude of the change in the calculated asymptotic fouling resistance with feedrate and shows the extent to which fouling is reduced at higher feedrates. For comparison, the values for fouling resistances calculated using data from various sources are shown in Table 3 which may give some indication of the effect of the choice of evaporator design on fouling. (28).

Tests at Felixton (FX) during 1995. Magnetic treatment

A device employing strong permanent magnets, manufactured in the USA, was tested at FX during the 1995 season. The evaporator train at FX consists of two parallel evaporator trains and the treatment was applied to one train only, the other being used as a control. Treatment was changed from one train to the other to avoid a systematic error since inherent differences may exist which are not obvious. The magnets were fitted by the factory staff, to the satisfaction of the suppliers, initially on the feed line to the A phase evaporator train, before the first effect; then later in

the season to the B phase before the 5th effect evaporator. Measurements of the clear juice and syrup Brix, and flowrates were used to calculate the heat transferred per unit area (Q/A) for the whole train. The results are shown in Table 4. This shows that the A phase evaporators outperformed the B phase evaporators but that performance was not significantly changed by using the magnetic device. Scale samples were taken at regular intervals from both trains. Examination of the scale composition using the methods described above showed no difference in the composition when "treated" and "untreated" scales were compared. Cleaning staff could detect no difference in the scale formed in the treated from that in the untreated evaporator/s. It can therefore be concluded that magnetic scale control devices do not have any effect on evaporator fouling at FX.

Tests to evaluate the effectiveness of antiscalent

The effect of chemical additions at Noodsburg (NB) and Union Co-operative (UC), both midland mills, were evaluated during the 1994 season and again at UC during the 1995 season. During 1994, at both mills, samples of scale were taken after "normal" operation and were analysed to establish the typical scale composition. Following antiscalent trials at UC and NB over a period of two weeks, samples were again taken and analysed. At both mills the antiscalent used was supplied as a commercial product, and was dosed at 10 ppm (g/ton) into the clear juice and 3rd effect respectively.

Following the antiscalent trials at NB, SO_2 was added to the juice for a two week period, at the rate of 200 ppm on Brix, to improve colour and viscosity. Scale samples were also taken to evaluate the effect, if any, of SO_2 on the scale formed.

Results of UC trials

Analysis of the UC scale taken under "normal" conditions during all trials reported here showed relatively little crystalline material to be present, with only ACP formed in the initial effects and COM and COD in the later effects. After running with antiscalent addition during the 1994 season, scale samples analysed showed the same phases present. However, XRD and HPLC analysis demonstrated quite clearly that the amount of crystalline oxalate deposited was reduced by about 30% by using the antiscalent. The operating staff at UC considered the antiscalent effective in that higher crush rates could be maintained during the test period than were normally

expected, and the scale was more easily removed (mechanically), although no HTC data were recorded.

More thorough testing over the entire 1995 season at UC involved the treatment of only one of its two parallel trains, as for the FX magnetic device evaluation tests. The results of the heat transfer measurements are given in Table 5, which shows no significant improvement in the heat transfer rates between the treated and untreated trains. However scale samples analysed showed again that the antiscalent effectively prevented the formation of calcium oxalate in the treated evaporator trains and mill staff reported that the scale formed was softer and very easily removed. These results suggest that the antiscalent used was effective at reducing the amount of crystalline scale formed, but did not make a significant change in the rate of heat transfer.

Results of NB trials

Scale samples from the third and fourth effects at NB were found to contain high levels of CAC with smaller amounts of COM and COD. When antiscalent was dosed into the first and third effects, samples of scale were still found to contain CAC. However, XRD and HPLC analysis indicated that levels of CAC were reduced by about 30% and only COM was found in very small amounts, i.e., it appears that the antiscalent prevents the formation of COD. However SO_2 appeared to be even more effective than antiscalents at reducing the amount of crystalline CAC deposited since the relative peak heights were reduced by about 60%. SO_2 appears to inhibit the formation of COD and a small relative increase in the amount of COM was noted, but overall the amount of oxalate formed was reduced. XRF analysis showed that amorphous silica was increased in the second, fourth and fifth effects when both antiscalent and SO_2 were used, the influence of the latter being greater than the former. Similarly the additives were found to reduce the relative amount of magnesium in the scale samples, again SO_2 being more effective than antiscalent. The observations can be explained in terms of both additives being effective complexing agents for calcium and magnesium but not for silica. Furthermore SO_2 is a reducing agent, dropping the redox potential, thereby preventing the formation of oxalate according to the theory of Walford (26).

In spite of these well defined changes in the scale composition, no change in the ease of cleaning of the evaporators was noted by the process staff in either of the tests at NB where additives were used. However it must be noted that the CAC formed at NB was so hard and difficult to remove that evaporator cleaning could not be completed

in the weekly shut down time available. Thus the presence of a layer of previously formed "old" scale may have swamped the effect of softer "new" scale that may, or may not have been formed more slowly. Since ease of cleaning (scale softening), or a clear reduction in the quantity of scale formed, is ultimately necessary to consider an antiscalent effective, the test was considered to be unsuccessful. Nevertheless, the analysis carried out showed its sensitivity to the changed conditions. These results suggest that to some extent the antiscalent was effective at controlling the crystalline deposits and scale hardness. It does not however indicate a change in the rate of deposition or the quantity deposited, and where the fouling is extensive the effect of even a large reduction in the crystallinity may be swamped by the total mass of scale formed, or inadequate cleaning between campaigns.

SUMMARY AND CONCLUSIONS

This work has shown that scale formed in Southern African mills follows the same trends reported in the literature from studies carried out in other countries, and techniques of scale reduction or removal are thus of interest to the cane sugar industry as a whole. Some anomalies appear to arise from time to time, but the analytical procedures developed provide an effective tool for identifying the major compounds formed and understanding the precipitation pathways leading to scale deposition. Techniques for evaluating possible methods of mitigating the effects of scale have been shown to be effective, although this work has highlighted the difficulties involved in making sound conclusions in terms of the requirements for a test to be considered successful. Results so far suggest that the most productive direction for further work in terms of reducing the effects of fouling in the short term may lie in the direction of modified evaporator design and operation. Eventually, demineralisation or decalcification will be required to eliminate evaporator fouling in the South African sugar industry completely. Antiscalents thus far appear effective at reducing scale hardness and preventing crystalline scale from forming but do not seem to prevent silica from depositing. Significant improvements in the rate of heat transfer have not been established using antiscalents. A magnetic device tested was found not to change the scale formed in any way, either in terms of heat transfer rates or in the scale composition.

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Table 1. Ratios used to calculate compounds in scale from analytical data.

Compound found in Scale	Form after ashing	Estimated LOI: mass lost /compound Mwt.
Silica $\text{SiO}_2 \cdot x\text{H}_2\text{O}$	SiO_2	$18/78 = 23\%$
$\text{MgO} \cdot \text{H}_2\text{O}$	MgO	$18/58 = 31\%$
Amorphous calcium phosphate $\text{Ca}_{1.5}(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ (ACP)	$\frac{1}{2}(\text{P}_2\text{O}_5) + 1\frac{1}{2}\text{CaO}$	$36/191 = 19\%$
Hydroxyl apatite $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$ (HAP)	$10(\text{CaO}) \cdot 3(\text{P}_2\text{O}_5)$	$18/1004 = 2\%$
Brushite $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (BR)	$\text{CaO} \cdot \frac{1}{2}\text{P}_2\text{O}_5$	$45/172 = 26\%$
Collensite (Calcium phosphate hydrate) $\text{Ca}_2(\text{Mg,Fe})(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ (CPH)	$2 \text{CaO} \cdot \text{MgO} \cdot \text{P}_2\text{O}_5$	$36/330 = 11\%$
Whewellite: calcium oxalate monohydrate $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ (COM)	CaO	$90/146 = 62\%$
Weddelite: calcium oxalate dihydrate $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ (COD)	CaO	$108/164 = 66\%$
Calcium magnesium aconitate $\text{Ca}_2\text{Mg}(\text{C}_6\text{H}_3\text{O}_6)_2 \cdot 6\text{H}_2\text{O}$ (CAC)	$\text{CaO} \cdot \text{MgO}$	$458/554 = 83\%$
Amorphous organic	$\text{CO}_2 \uparrow$	100%

Table 2. Scale analysis from some Southern African Mills during the 1995 season demonstrating the results obtained using the procedure described, and the agreement between the LOI estimated from the calculated composition and that obtained experimentally. "R" refers to Roberts vessels and "K" to Kestner or semi-Kestner vessels. Scales from MS show the variation over time of the crystalline phosphates.

Mill & Date	Vessel and Effect	Phosphate		MgO. .H ₂ O	SiO ₂ . .H ₂ O	All Oxalate	"Lime" Hydr /carb	Amorph. Organic	LOI	
		Crystalline	ACP						Est.	Meas
MS. 11/07/95	K(1st)	18 [CPH]	37	20	3	1	0	20	41	41
	R(2nd)		19	3	5	15	25	34	50	54
	R(3rd)		6	1	4	39	32	38	52	59
	R(4th)		9	0	39	22	16	13	40	47
	R(5th)		22	6	35	21	3	13	44	40
MS. 18/07/95	K(1st)	11[HAP+BR]	37	6	12	0	3	28	44	39
	K(1st)	1 [HAP]	41	6	12	1	0	24	40	39
	R(2nd)		20	2	2	39	23	33	56	39
	R(3rd)		6	1	6	17	56	33	58	57
	R(4th)		6	0	31	15	27	22	47	47
	R(5th)		26	3	27	10	33	1	26	42
MS. 25/07/95	K(1st)	19[HAP]	36	4	19	4	0	21	36	39
	R(2nd)	1 [HAP]	13	2	3	17	27	40	58	59
	R(3rd)		3	0	6	15	33	39	61	31
	R(4th)		7	1	34	20	26	13	41	41
	R(5th)		19	0	27	33	13	21	46	43
UR - 4/7/1995 (Swazi- land)	R(3rd)	0.4 [HAP]	8	2	19	4	21	40	62	57
	R(3rd)	2 [HAP]	43	6	12	4	2	21	40	41
	R(4th)	1 [HAP]	19	3	12	1	26	17	40	39
	R(4th)	1 [HAP]	19	2	35	1	26	25	46	41
GD Dec 94	R(1st)	24 [HAP]	27	3	1	0	1	44	51	47
	R(2nd)		36	4	7	1	13	38	48	46
	R(3rd)		1	0	3	60	13	17	52	57
	R(4th)		1	0	28	40	16	16	52	57
ML end 95	1st		3	1	23	17	1st	36	58	58
Malawi Jun 95	K(1st)		43	4	43	1	0	1	39	28
	K(1st)		43	4	44	4	0	17	31	31
	R(2nd)		41	4	44	1	0	39	30	29
	R(3rd)		37	3	21	2	0	36	50	48
	R(4th)		39	4	44	5	26	0	28	29
	R(5th)		18	2	64	5	15	0	25	31

Table 3. Fouling resistance values for various evaporators.

Mill	Evaporator type	Estimated fouling resistance (m ² °C / kW)	Reference
SZ	1st effect Kestner	2,0	22
PG	1st effect Kestner	0,4	23
PG	1st effect Falling film	0,3	23
GD / Australian mill	2nd effect rising film	0,1	28
	Plate evaporator		12
FX Main Evap.	2nd effect	0,2 - 0,3	30
FX pilot plant	2nd effect simulation	0,1-0,3	30

Table 4. Results of magnetic treatment trials at the FX sugar mill. Values are heat transferred per unit area with units in kW/m².

	A Phase		B Phase	
Magnets	on	off	on	off
Average	10,6	10,7	8,7	9,5
Standard deviation	1,66	2,02	1,82	1,31
No. of measurements	188	124	113	199
Maximum	13,2	14,2	12,3	11,4
Minimum	7,1	2,2	2,2	5,5

Table 5. Results of antiscalent trials carried out at UC during 1995. Values shown are heat transferred per unit area (kW/m^2).

	Train 2		Train 1	
	Treated	Untreated	Treated	Untreated
Average	19,4	19,5	18,8	18,5
Standard deviation	1,4	1,4	1,1	1,1
Maximum	22,3	22,6	20,6	20,7
Minimum	17,1	15,9	14,1	15,2
No of measurements	49	94	66	77

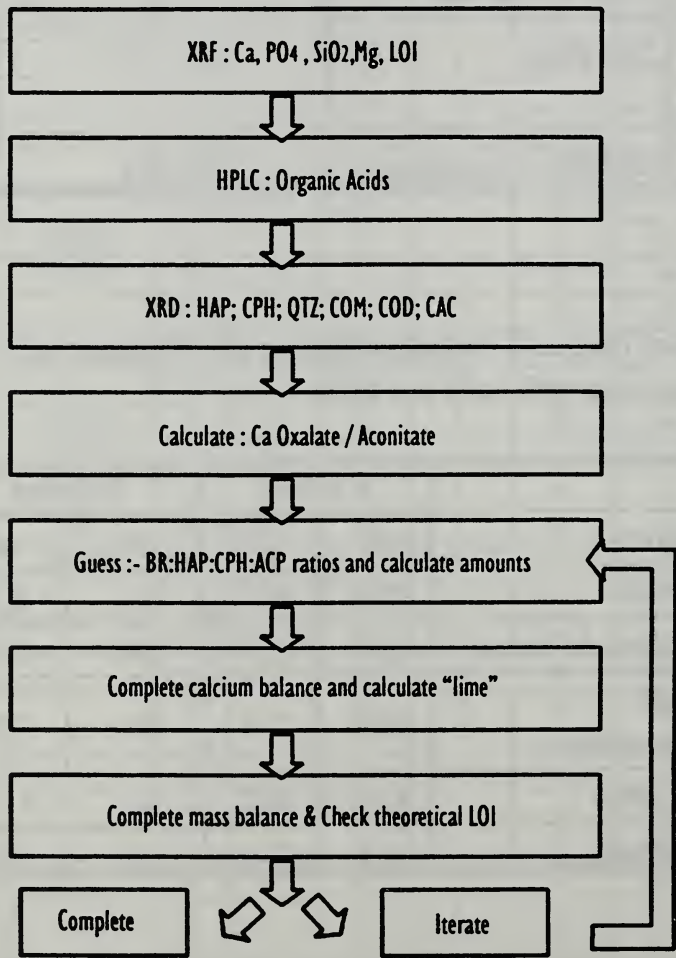


Figure 1. A general procedure for the analysis of evaporator scale.

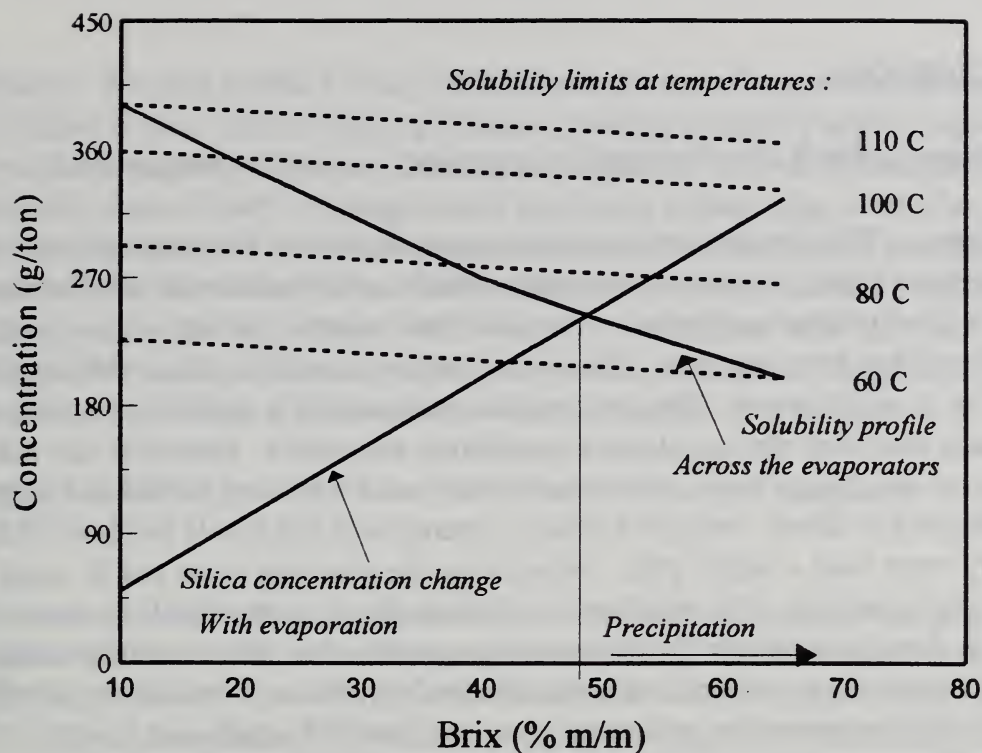


Figure 2. Precipitation of silica, assuming a solubility mechanism.

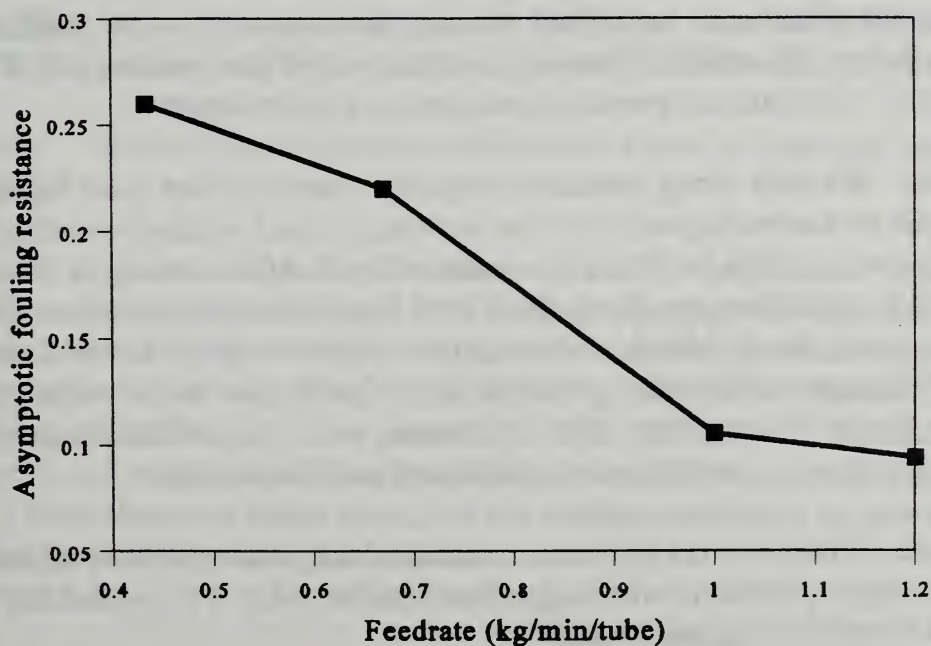


Figure 3. The change in asymptotic fouling resistance with varying flowrate; data obtained using a long tube climbing film pilot evaporator.

DISCUSSION

Question: Which cleaning/washing system do you use for evaporators?

Walthew: The cleaning system varies among factories. For example, one factory (in Northern Natal) cleans out every three weeks, while another in a different region (North Coast) cleans every week. The mill where we ran the anti-scalant trial cleans whenever they have any stop: There is a team on standby to clean the evaporators. There is no single system. The fact that the heat transfer is declining over the season indicates that they are not always completely successful. Factories can not really clean on an ad hoc basis. They must clean on a Monday (scheduled stop day). Cleaning is a problem - being the slowest operation in the whole factory. The entire factory must wait - which puts pressure on the cleaning team and it could cause incomplete cleaning. The solution is to reduce the amount of scale formed.

Only three factories use chemical cleaning agents - they all use caustic soda. They are a bit cautious to use acid because of fear of corrosion. There are many different metals in the evaporators, and many opportunities for corrosion in South African factories.

Question: Two questions regarding magnets: There are some indications that magnets are effective in very dilute streams, for instance in water purification, in streams below 1% solids. What concentrations were you working at? The other question is: Did you use permanent magnets or electromagnets?

Walthew: We used strong, permanent magnets. There has been some testing at one of the mills on electromagnets, but I can not discuss that because the method of data collection was unreliable. There is a group in South Africa looking at this subject: They will be publishing very shortly (Ref: Prof. Paul Gettree, Rand African Universal, Johannesburg, South Africa). With regard to dilution: Input to the first effect is about 10 Brix; to the fifth effect, about 50 Brix. On the question of water treatment, a large South African water firm has tested, not a permanent magnet, but an electromagnet, on a parallel system, and found no effect to date.

Question: When there is a high concentration of magnesium before evaporation, as with the Magox process, or with magnesium bisulfite, what is the probability that we will find a scale of magnesium salts?

Walthew: We have looked at that, and I believe the use of magnesium is desirable. The problem is cost. There is also a problem in creating a suitably active magnesium. The temperature must be right in activating the magnesia - you do not want to create an insoluble phase. There is a danger that if calcium is present also, a lot of aconitate scale could be made. The calcium magnesium aconitate solubility is concentration dependent. On the one hand, the medical solution to calcium oxalate formation (as kidney stones) is to take magnesium because it complexes with oxalate. In the factory, if there is significant aconitic acid present, addition of magnesium could increase the precipitate. This needs experimental testing.

Question: Would you say that sulfate is not an important component to scale in South Africa?

Walthew: All factories in South Africa use defecation, (that is, phosphate precipitation). We do not use sulfitation. To explain about the SO_2 we used: That was put in as a trial to control color and viscosity. It was just opportunistic that we could measure the effect. If there is a lot of sulfate (e.g. from sulfitation) in juice, then gypsum (calcium sulfate) will be a big problem in scale. Gypsum is a difficult scale to deal with, because it is not very soluble in anything. The antiscalant people claim to be making great strides in that area. Our problem with antiscalants is that to get an effect, quite high concentrations of antiscalant must be used, and these concentrations may exceed FDA limits. Or, a non-acceptable chemical has to be used.

Question: What do you anticipate will be the future in chemical antiscaling compounds?

Walthew: I am not in a position to answer that. However, the new antiscalants seem to be versions of old antiscalants - the problem is getting FDA approval. There is a need - there is a big opportunity here - for some fundamental research in that area. The Australians seem to be doing this, precipitating scale under various conditions and observing the effects of various antiscalants. The problem with manufacturers is that they do not want to reveal the composition, so you have to use the product on trust. I think there is a future there, but the ultimate solution is to prevent scale formation by removing calcium from juice.

CHEMOMETRICS FOR PROCESS AND PRODUCT CONTROL IN THE SUGAR INDUSTRY

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ABSTRACT

The powerful combination of spectrofluorometry and chemometrics on sugar process samples is demonstrated by three examples: A) The basis for a new fast method for the quantitative determination of amino-nitrogen in beet brei is developed. The optimal method is found by the selection of excitation/emission wavelength pairs by means of the principal variables algorithm. B) The potential in utilising classical chromatographic separation of different process juices/liquors/syrups in combination with spectrum-producing detectors is outlined by the analysis of a molasses sample. C) Finally, a preliminary concept of total process monitoring is given on the basis of spectrofluorometric analyses of twenty-five different process juices ranging from the diffusion juice to the molasses.

INTRODUCTION

White sugar made from cane or beet contains very small amounts of impurities which can be detected by spectrofluorometric analysis (2,8). In an earlier paper (8) on chemometric evaluation of fluorescence spectroscopy and sugar quality data from a white beet sugar production we have demonstrated how chemometrics can enhance the information content dramatically when dealing with such data. The two primary conclusions were: A) White sugar samples and, to a certain degree, thick juice samples can be classified according to factory site by analysing the spectral fingerprint produced by the sugar/thick juice samples or by analysing the quality data alone. B) It was possible to predict the most important quality parameters, e.g., colour, ash%, amino-nitrogen, and SO₂, of both white sugar and thick juice from the spectral measurements on these products.

In this paper we will present a series of chemometric applications in different stages of the beet sugar production chain ranging from the sugarbeet brei to the final product sugar and the by-product molasses. First, the basis for a new, extremely fast method for the determination of amino-nitrogen in sugarbeet brei is developed. Then, we will discuss the chromatographic separation of different process juices/liquors/syrups and demonstrate how to utilise the large amount of data produced by such chromatographic systems when combined with spectrum-producing detectors. The potential is demonstrated by the analysis of a molasses sample. Finally, a preliminary concept of total process monitoring based on spectrofluorometric analyses of all different kinds of process juices from the diffusion juice to the molasses is presented.

METHODS AND MATERIALS

Samples

Double ion exchanged water is used for all solutions and dilutions.

Beet brei samples. Twenty-four beet brei samples are processed according to the $\text{Al}_2(\text{SO}_4)_3$ -method in order to achieve a clarified solution.

Molasses. The molasses sample is diluted 1:100 prior to analysis. One ml of the diluted sample is put on a PD10 column (Sephadex G25) and eluted with water. Seventy-seven fractions of four drops each are collected. Each fraction is further diluted with 2 ml of water before fluorescence analysis.

Total process monitoring samples. Twenty-five samples from the whole sugar process are collected, including diffusion juice, pre-limed juice, hot and cold main lime juice, thin juice, 1st carbonatation, 2nd carbonatation, 3rd effect, thick juice, white 1 (first product), white 2 (second product), sugar, standard liquor 1-3, melt liquor, massecuite 1-3, affination syrup, green syrup 1-2, wash syrup 1 and 3, and molasses. Dilution experiments are performed with all samples in order to ensure that the fluorescence spectra measured are all in the non-quenched area (8).

Spectroscopic measuring conditions

Fluorescence emission spectra are recorded on a Perkin Elmer LS 50B spectrometer connected to an IBM-compatible PC. All calculations are performed with Matlab for Windows version 4.2c.1 (MathWorks, Inc.) and Unscrambler version 5.5 (CAMO

A/S). See references 8-10 for further details about software, including software for instrument control.

All measurements are performed with a maximum scan velocity of 1500 nm/min, and with both excitation and emission monochromator slit widths adjusted to 10 nm. The sample holder is thermostatted to 24°C ± 0.1.

Beet brei experiment. Emission spectra are recorded at excitation wavelengths of 230 nm, 240 nm, and 340 nm. The emission ranges sampled at 0.5 nm intervals are 275-560 nm, 275-560 nm, and 363.5-560 nm, respectively (a total of 1536 data points per sample).

Separation experiment (molasses). Emission spectra are recorded at excitation wavelengths of 230 nm, 240 nm, 290 nm, and 340 nm. The emission ranges - all sampled with 0.5 nm intervals - are 275-560 nm (a total of 2042 data points per sample after removal of Rayleigh scattering peak (9)).

Total process monitoring experiment. Emission spectra are recorded at excitation wavelengths of 230 nm, 240 nm, 290 nm, and 340 nm. The emission ranges all sampled with 0.5 nm intervals are 275-560 nm (a total of 2036 data points per sample after removal of Rayleigh scattering peak (9)).

Chemical measurements

Chemical measurements of amino-nitrogen (amino-N, ppm) and colour are performed. The colour measurements are performed in accordance with the International Commission for Unified Methods of Sugar Analysis (ICUMSA). The amino-nitrogen content of the beet brei samples was determined by a standard autoanalyser instrument based on the ninhydrine method.

Chemometric methods

For a thorough explanation of the chemometric methods of principal component analysis (PCA) (3,7,12), partial least squares (PLS) (3,4,7), and principal variables (PV) (5), the reader is referred to the literature. In this context PLS is used to make predictions of the traditional chemical analysis amino-N from the rapidly acquired fluorescence spectrum. The root mean square error of prediction (RMSEP) is used for the evaluation of the prediction error and is defined as

$$\text{RMSEP} = \sqrt{\sum_{i=1}^N (C_i^{\text{Predicted}} - C_i^{\text{Reference}})^2 / N}$$

where $C_i^{\text{Predicted}}$ is the model estimated concentration, $C_i^{\text{Reference}}$ is the reference value, and N is the number of samples.

In order to determine the optimum number of PLS-components in the prediction models the method of full cross validation is applied (7,11): One sample is excluded from the data set and a calibration model is built on the remaining samples; next, the amino-N value of the excluded sample is predicted, using the spectral measurement as input to the developed model, in order to evaluate the prediction error. This cycle is repeated for all the samples, and a total RMSEP is determined for each PLS-component.

RESULTS AND DISCUSSION

Amino-N in sugarbeet brei

Twenty-four samples of sugarbeet brei extracts were measured to give the full emission spectra at three different excitation wavelengths: 230 nm, 240 nm, and 340 nm. Corresponding amino-N values were obtained from a classical autoanalyser based on a ninhydrine reaction. This type of analysis includes the use of hazardous chemicals and solvents and the maximum number of analyses per hour is approx. 80. In order to avoid the hazardous chemicals and in order to achieve a fast method of analysing the amino-N content, it is desirable if an at-line analysis of amino-N by fluorescence spectroscopy can substitute the traditional instrumental analysis. This will make it possible to monitor the amino-N content continuously. The concatenated raw emission spectra of the 24 beet brei samples are shown in Figure 1. A full spectrum PLS regression of all 1536 wavelength variables versus amino-N gave a correlation of 0.96 and an RMSEP of 14.5 based on three PLS-components (see Figure 2). The prediction errors are found by a full cross-validation of the PLS-model. Samples with low amino-N values have relatively large prediction errors and the RMSEP is reduced by 37 % by exclusion of these samples. In all subsequent models all samples are included, as this is the real situation.

The spectral analysis was performed with a research instrument not suited for at-line/on-line purposes. It will be investigated if it is possible to obtain an equally low prediction error with a strongly reduced set of wavelength variables (of the order of 3-5 excitation/emission wavelength pairs), since instruments for such limited cases are

already developed for industrial purposes. Another scientifically interesting aspect which may be investigated on the research spectrofluorometer is the comparison of the spectral information at each excitation wavelength, and the question if a combination of the information from all three excitation wavelengths is necessary to get a prediction model equal to the full-spectrum model. The latter is explored by making three full cross-validated PLS-models based on the spectral input from each of the excitation wavelengths. Table 1 outlines the results from this analysis. The three possible combinations of information from pair-wise excitation wavelengths are investigated. The variables selected by principal variables (PV) among all wavelengths correspond to the ones one would select by visual inspection of the data, namely the variables at the three emission peaks at each excitation wavelength. PVs and regression models for these are also found for the combination of excitation wavelengths of 230 nm and 240 nm and for excitation 230 nm alone. Finally, the best univariate regression model among all possible 1536 models is calculated. All results are collected in Table 1 for comparison.

We observe that the lowest prediction error is obtained by utilising all variables or only variables from excitation 230 nm and 240 nm in combination, indicating that the information at 340 nm is not as relevant for amino-N predictions. However, the spectral information at 340 nm is different from the information at 230 nm and 240 nm since the full spectrum model uses three PLS-components, while the reduced model only uses two dimensions. It appears as if the relevant information regarding correlation to amino-N increases with decreasing excitation wavelength, as seen from the results for the individual excitation wavelengths.

By selection of variables among all variables (or among variables from excitation 230 nm and 240 nm) a slightly larger prediction error is obtained. In addition, it is interesting that it is possible to use only two variables to obtain a very low prediction error. Using only excitation 230 nm the model needs five principal variables in order to give a satisfactory model. From an instrumental point of view the latter result is important since only one excitation filter is necessary which makes the measurement extremely fast (<20 seconds per sample).

It is concluded that it is possible to develop a fast industrial at-line instrument specifically for the analysis of amino-N in sugarbeet brei. The calibration model only needs updating by means of calibration to the traditional analysis. A subject not treated here, but which certainly is of great importance, is how often it is necessary to recalibrate the system.

Qualitative analysis of thick juice and molasses

It is crucial to make both a qualitative and quantitative analysis of the high and low molecular compounds present in all types of process juices/liquors/syrups, when discussing the mechanism of colour formation in the sugar production chain (6) so vital to the industry. Preliminary investigations in this area have been performed by the analysis of a molasses sample.

The experiment conducted was a very simple Sephadex G25 separation of high and low molecular compounds of molasses. The raw spectral data for all 77 fractions are shown in Figure 3. It is obvious that the spectrum corresponding to fraction No. 3 has extremely large intensities. This spectrum is removed in the further analysis, as it is assumed to be erroneous. Two PCA score plots of the remaining 69 fractions are seen in Figure 4. The variance described by the first four principal components is 68.35%, 17.90%, 12.06%, and 1.12%, respectively (a total of 99.43%). A certain migration trend is seen in the score plots, representing the spectral changes of the elution profile. Using PCA on the raw spectra gives the numerically large spectra a strong influence on the final model. In order to put the fraction spectra on an equal scale the raw spectra are normalised (the sum of the squared elements of each spectrum is then equal to one) before running the PCA decomposition. The PCA results from this analysis are shown in Figure 5. The first fractions up to No. 40 (represented by Nos. 20 and 40, see Figure 6A) are the coloured high molecular melanoidines and melanines displaying spectral information in the visible and in the visible-UV region. By studying the score plots at least two additional special fractions are revealed corresponding to Nos. 47 and 63, which are representative to the precursors of colour displaying spectral emission information at low excitation wavelengths. The normalised spectra from these fractions are also shown in Figure 6A. In future studies it will be interesting to qualitatively compare these fraction spectra with pure substance library spectra. For comparison purposes the corresponding spectrum of dihydroxyphenylalanine (DOPA) is shown in Figure 6B. A high degree of similarity between fraction No. 47 and DOPA is seen, indicating the presence of this substance in the molasses. It is observed that the Sephadex G25 separation also is able to separate the low molecular compounds. It is well known that Sephadex separation is delayed in aromatic amino acids and phenols.

The long-term purpose of the above investigations is to be able to collect full 3D-landscapes (full emission spectra at, for instance, 30 excitation wavelengths) several times each minute during the chromatographic elution. By analysing 30-40

samples in this way, it is most likely that it will be possible to make a calibration model based on a spectrofluorometric measurement of the diluted raw thick juice and the quantitatively determined components in the chromatogram. In this way, one will have a fast at-line estimate of the different colour components in the process juice in question. The chemometric analysis of this pattern will certainly reveal interesting aspects about the status of the process and how the different process stages are connected with respect to the complex problem of colour formation. Such analyses are in progress at our laboratory (1).

Total Process Monitoring

From an operator's point of view it is important to have a general view of the status of the total sugar production chain. This can be attained as illustrated in the following. Fluorescence spectra are measured on a number of process juices/liquors/syrups and the final white sugar (in this case twenty-five samples). A simple PCA on the normalised spectra reveals the current status of the process. In Figure 7 the corresponding score plot is seen. Two different areas are observed in the plot covering juice purification (upper left part) and the remaining juices. In the enlarged plot two groups are seen: the samples in the upper right part are input to the crystallisation of sugar, while the samples in the lower left part are in proximity to the final product. Using the measured colour of each of the process samples as a marker in the score plot (Figure 8) we see that the low, medium and high colour samples form four groups, two of which are medium colour groups. If we imagine that twenty-five on-line spectrofluorometers are situated in the process chain we will be able to get a score plot like the one in Figure 7 every fifth minute and in this way a special situation will come to view in the way the samples are placed in the score plots. Furthermore, the individual process juices can be monitored separately in order to focus on a local problem.

Conclusions

In an earlier study (8) it has been demonstrated that fluorescence spectroscopy and chemometrics is a very powerful combination for use in analysing patterns as well as specific components throughout the sugar production chain. In this paper we have enlarged the perspective of this combination by the development of a new fast method for the analysis of sugarbeet brei as well as by outlining the way classical separation techniques such as LC and HPLC can be applied for optimum information output. Finally, we have indicated a way of monitoring the whole sugar process by analysing

samples from every stage in the process and projecting all these analyses on the same two-dimensional pattern recognition subspace as an interface of information for the sugar process engineer.

ACKNOWLEDGEMENTS

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Table 1. Prediction errors (RMSEP) and correlation coefficients (R) for the prediction of amino-N in beet brei from fluorescence spectra. The regression models are based on a different number of spectral input variables.

Wavelength range	# variables	# factors	RMSEP	R
All	1536	3	14.5	0.96
All (without samples 1,8,18)	1536	3	9.2	0.98
Ex. 230 and ex. 240 nm	1142	2	14.5	0.96
Ex. 230 and ex. 340 nm	965	4	16.2	0.95
Ex. 240 and ex. 340 nm	965	3	27.0	0.85
Ex. 230 nm	571	5	15.5	0.95
Ex. 240 nm	571	4	31.1	0.81
Ex. 340 nm	394	5	37.8	0.74
Principal variables/selected from all variables ^{a)}	3	-	15.7	0.95
Principal variables/selected from ex. 230 nm and ex. 240 nm ^{b)}	2	-	15.7	0.95
Principal variables/selected from ex. 230 nm ^{c)}	5	-	15.5	0.95
Optimal univariate regression ^{d)}	1	-	26.3	0.86

^{a)} Variables 745, 170, 1270; corresponding to ex/em: 240nm/361.5nm, 230nm/359.0nm, 340nm/424.5nm (in selected order)

^{b)} Variables 745, 170; corresponding to ex/em: 240nm/361.5nm, 230nm/359.5nm (in selected order)

^{c)} Variables 169, 69, 297, 125, 367. Excitation wavelength throughout is 230 nm; emission wavelengths are 359.0 nm, 309.0 nm, 423.0 nm, 337.0 nm, and 458.0 nm (in selected order)

^{d)} Variable 318; corresponding to ex/em: 230nm/433.5nm

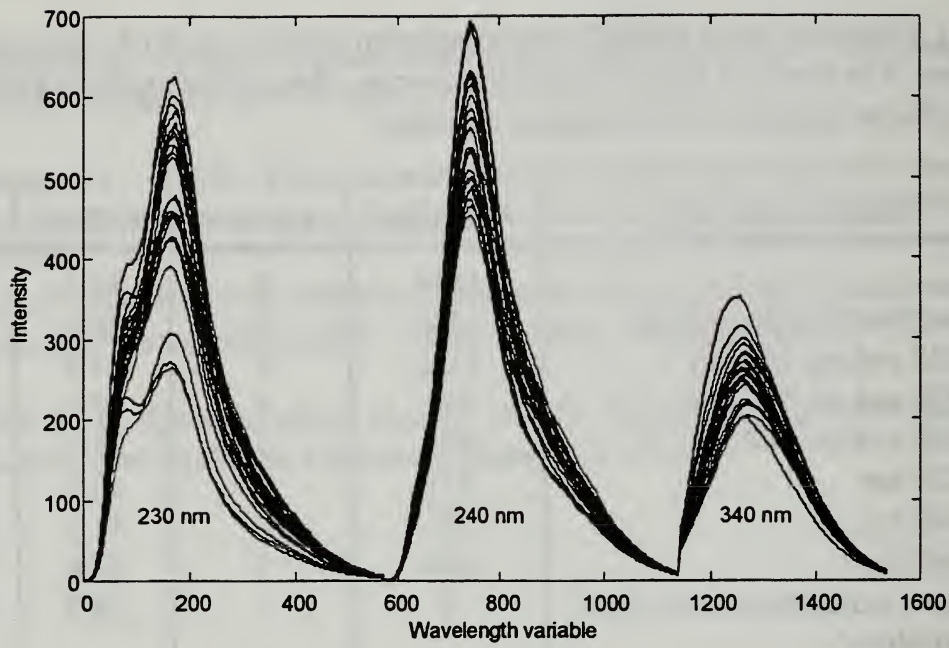


Figure 1. Fluorescence raw emission spectra of 24 sugar mash samples.

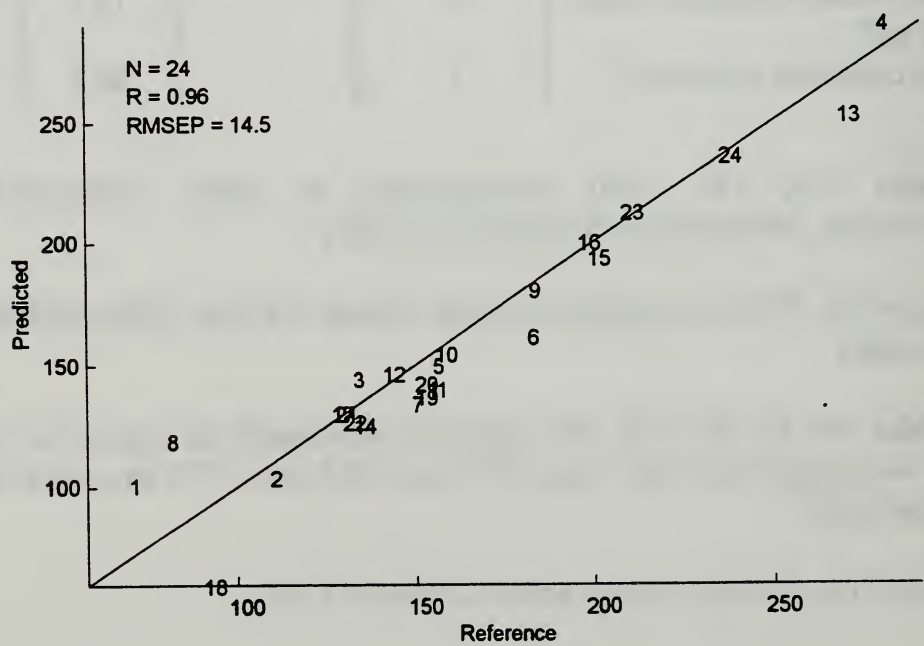


Figure 2. Predicted versus measured plot of amino-N values. Based on a 3 factor PLS-model with fluorescence spectra as independent variables and amino-N as the dependent variable.

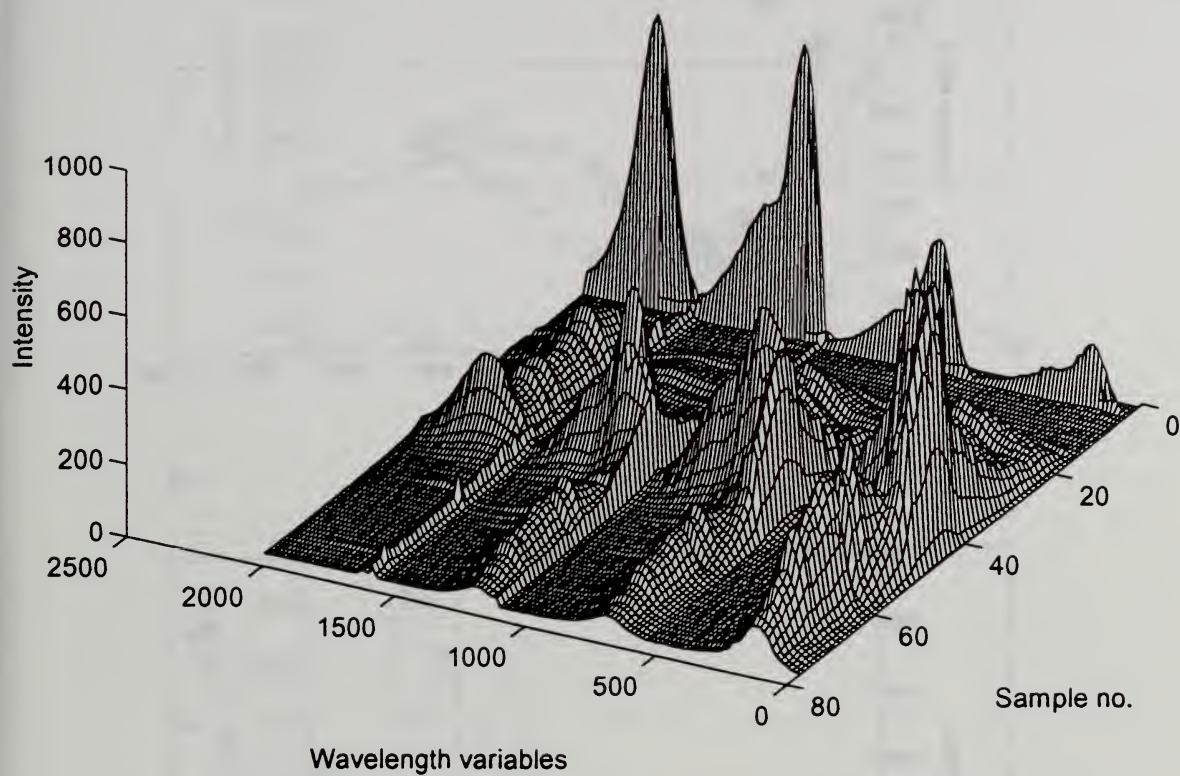


Figure 3. Fluorescence emission spectra of 77 molasses fractions. Sample 3 with the extreme high spectral signals is an outlier.

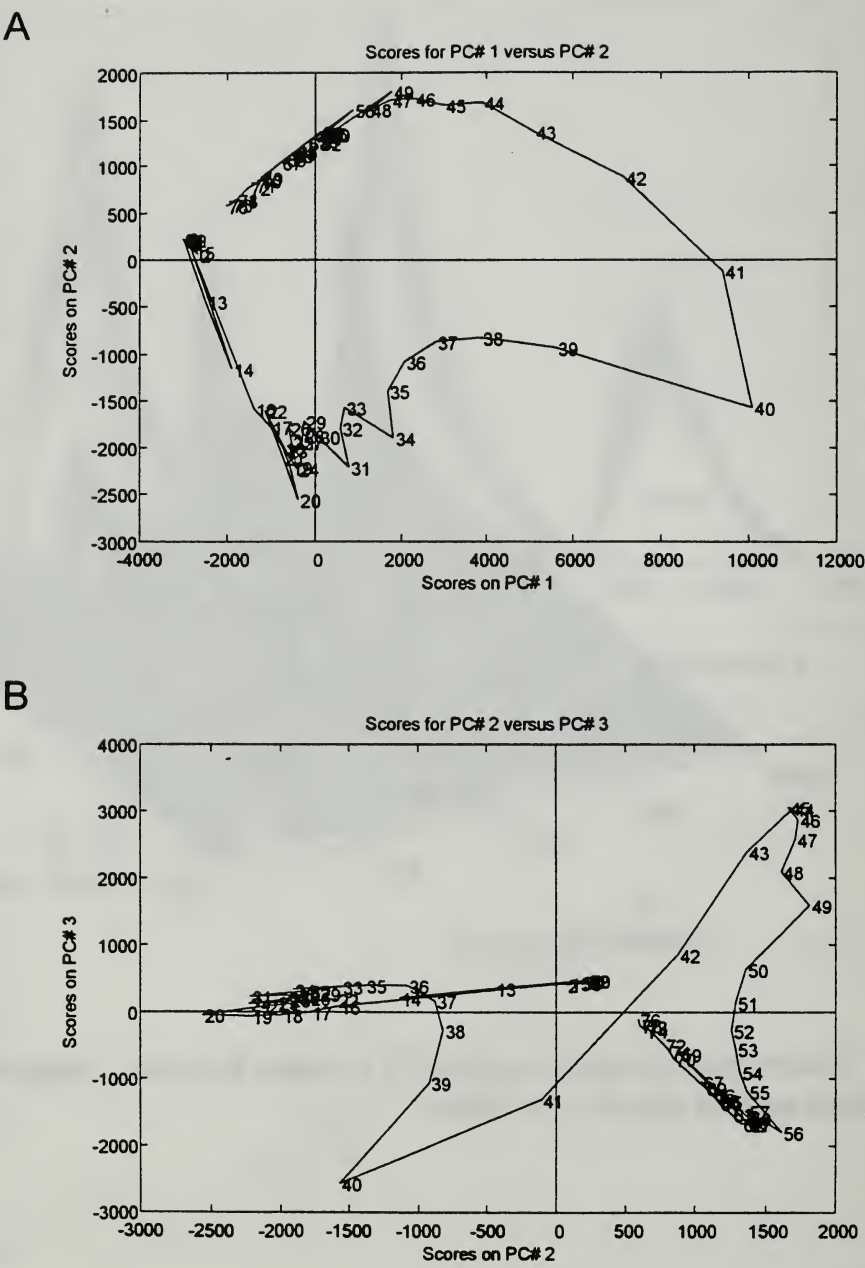
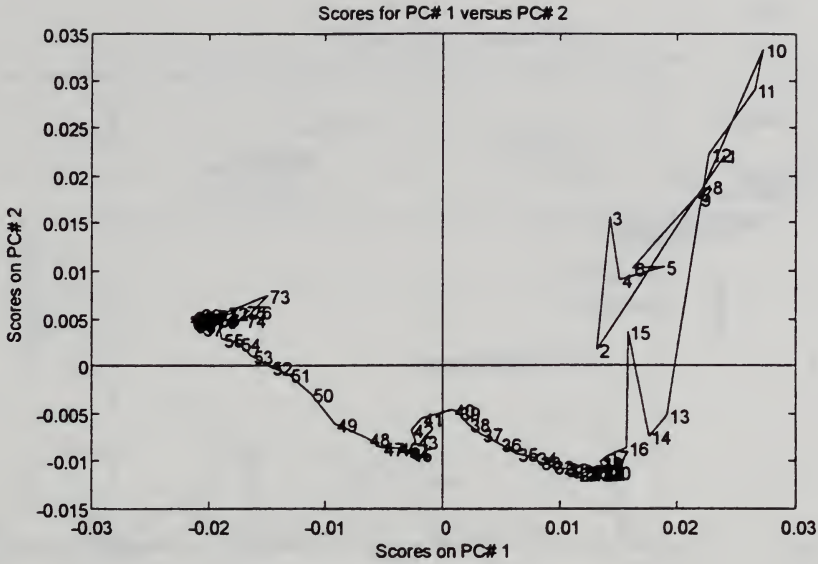


Figure 4. PCA on raw molasses spectra (sample 3 excluded, the remaining samples are renumbered from 1 to 76). A) Score one versus score two. B) Score two versus score three.

A



B

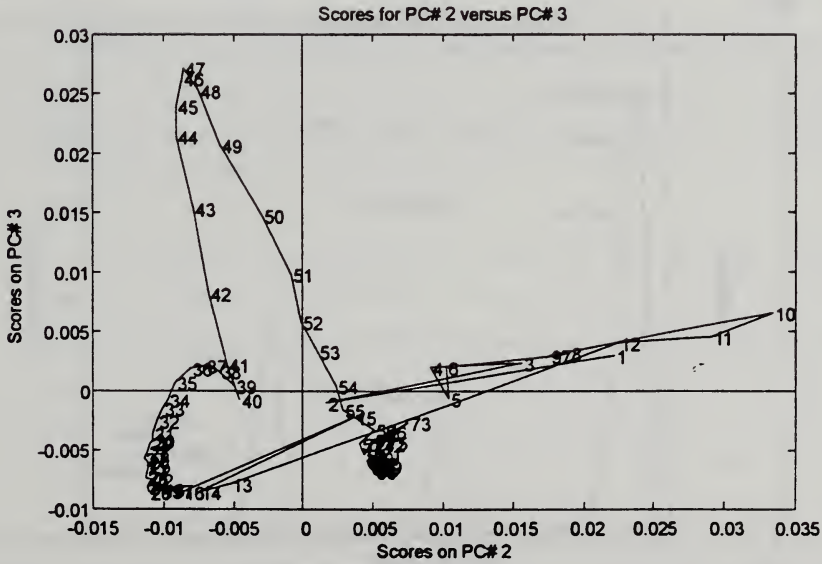
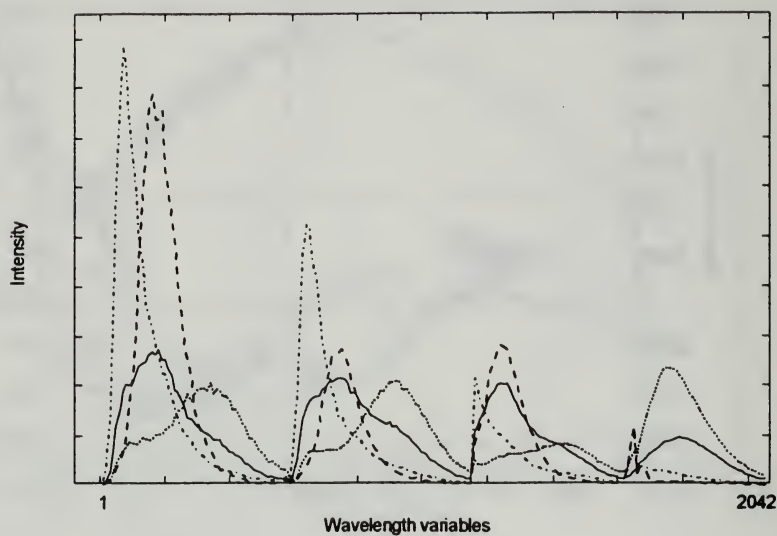


Figure 5. PCA on normalised molasses spectra (sample 3 excluded, the remaining samples are renumbered from 1 to 76). A) Score one versus score two. B) Score two versus score three.

A



B

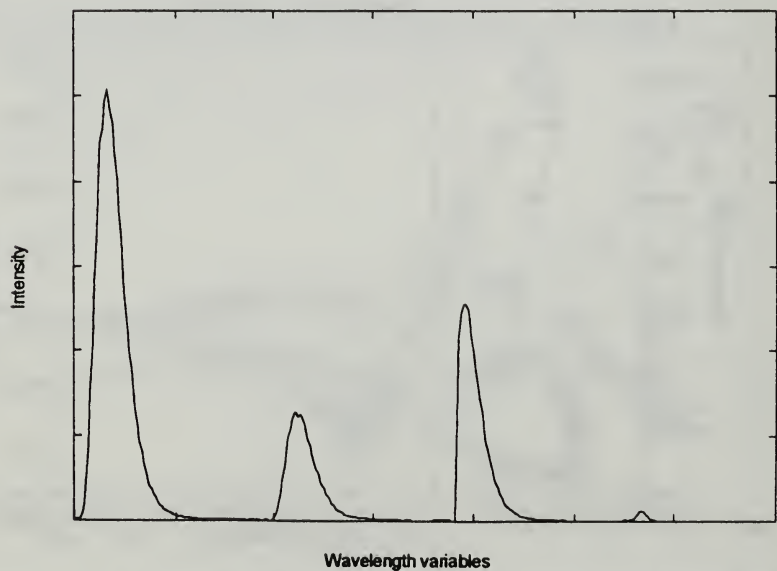
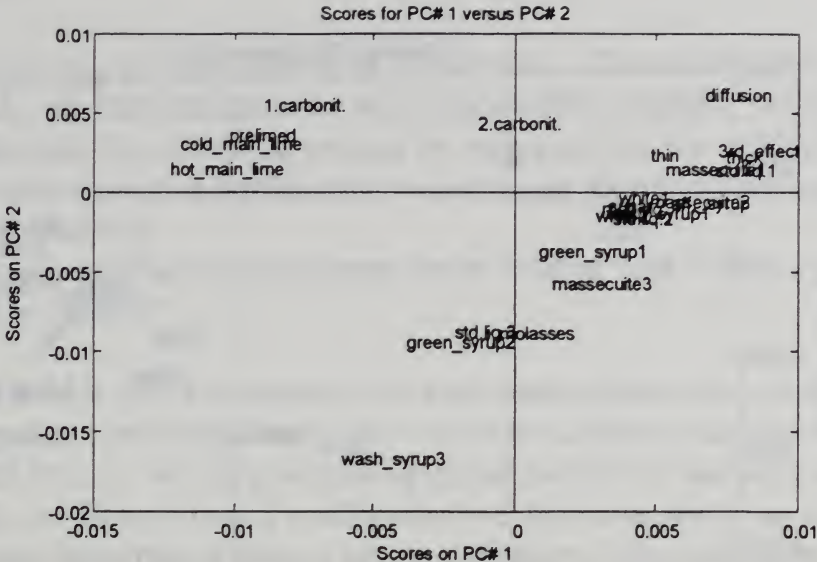


Figure 6. A) Normalised fluorescence spectra from times 20 (dotted), 40 (solid), 47 (dashed and dotted), and 63 (dashed). B) Fluorescence spectrum of dihydroxyphenylalanine (DOPA).

A



B

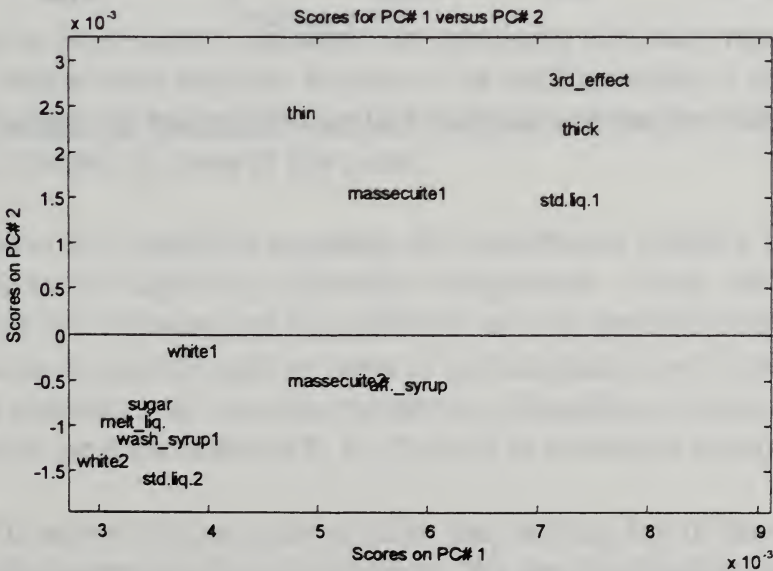


Figure 7. PCA on process samples. A) Score one versus score two. B) Enlargement of the right part of A.

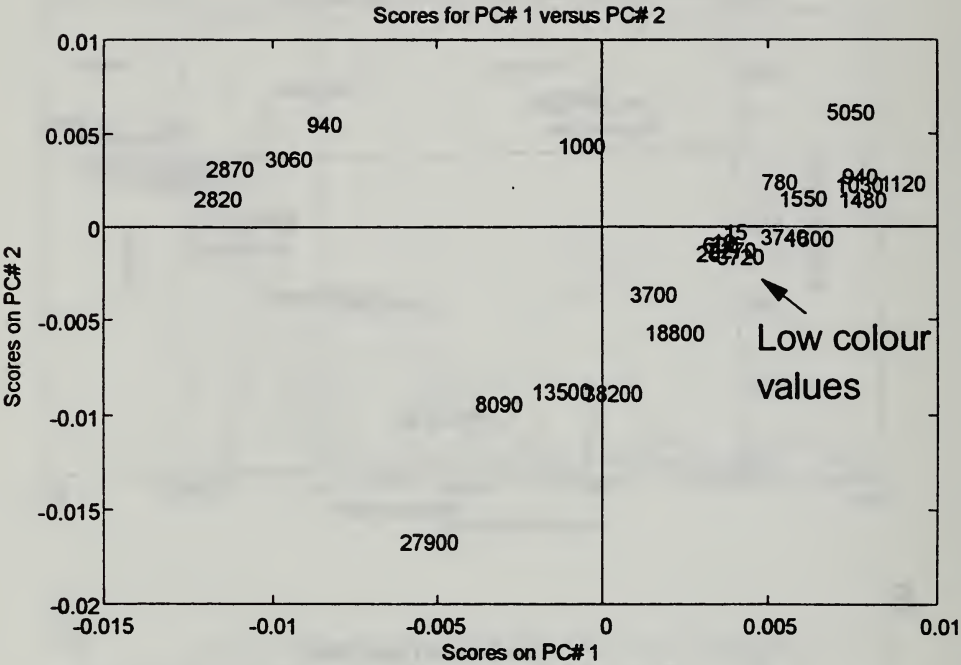


Figure 8. PCA on process samples. Colour values used as marker.

DISCUSSION

Question: Thank you for your interesting presentation. The methods on principal components look very much like those for near infrared (NIR) analysis. When an NIR method is developed, the calibration set may be very good but the validation in the next campaign may show that recalibration is necessary. Have you any experience with this sort of situation?

A second question: What are the costs in terms of labor, and of instrument cost, to develop such a method?

Nørgaard: To answer your first question: we have been collaborating with Danisco Sugar Development Center for three years. We have collected samples for those three years, and are now working on making global models for the different years. So, at this point, we do not know if a calibration will hold over the three years. We do know it is very important to make a proper validation of the calibration. Within the NIR field, these methods are widely accepted. Chemometrics almost evolved within the area of NIR technology, but, as you can see, it can be used to analyze all sorts of data.

In answer to your second question: we are doing the basic research, at the University, to make these methods function. At the University, I can go to the instrument manufacturer and explain how the method we have developed works. I can not give you details on costs at this point.

Question: I have two questions regarding the discriminant analysis, identification of factory source of sugars by principal components. First, how far apart geographically are the factories, and how different are the manufacturing processes? Are the differences in results based on beets or on factories, in your opinion?

The second question: you have shown only two dimensions in your discriminant analysis. Can you separate factories B, C, D and E in a third (or more) dimension?

Nørgaard: To answer the last question first: yes, we can, but of course it is very difficult to visualize more than three dimensions. We can discriminate among the six (two German and four Danish) factories by the SIMCA method, with an error of approximately 5%.

To answer your first question: what we see is the input, the sugarbeets, superimposed upon the action of the process. So we see a combination of both beet input and factory performance effects.

As to the distance: both factories in the presented example were in Denmark. As you know, Denmark is a small country, so they are relatively close.

Question: Have you tested sugars from other countries?

Nørgaard: We have tested factories from four factories in Denmark and two from Germany.

Comment: This is a paper about a system which we may all be using in ten years time.

PROPOSAL OF A NEW CRITERION FOR BEET SEED BREEDING

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ABSTRACT

The traditional criteria adopted by beet seed breeders for the evaluation of beet quality are critically reviewed. Such criteria could be modified by appraising the characteristics of the hot extraction juice obtained from micro-cossettes coming from individual beet samples. Such a juice is prepared via a system reproducing conditions which are comparable with those in industrial diffusion plants. The evaluation of the purity of such a juice might allow the breeders to know in advance the behavior of the various cultivars under the actual conditions in a sugar factory. Of course, evaluation of the sugar content, as well as dry matter and soluble and insoluble organic nitrogen contents of the beet, are to be carried out.

From the analytical point of view, it is essential to utilize NIR methodology, bearing in mind the problems arising from the employment of the traditional clarification agents, such as lead salts, and future environmental concerns.

INTRODUCTION

Breeding of beet seed is obviously based on the evaluation of the quality of the product and this must be considered from both agronomic and industrial aspects. In fact, it is necessary that beets present good productivity characteristics, considered as the yield of sucrose per hectare, as well as good characteristics related to the tonnes of crystallizable sugar per 100 tonnes of sugar entering the factory.

It is therefore extremely important to choose the correct method of beet quality evaluation. This should not only give as much reliable data as possible, but also data obtainable via rapid, computer-based, non-polluting analytical systems.

From the agronomic point of view, quality is assessed from the weight of the roots together with their sucrose content determined on the root brei via polarimetry. The system utilized is now highly automated with good reproducibility of the

polarization results and it is possible to analyse a considerable number of samples per hour. However, there are some negative points, i.e.:

1) as far as the polarimetric determination is concerned, the brei is traditionally mixed with basic lead acetate solution which after filtration gives a clear and practically colourless aqueous extract suitable to be read polarimetrically. Disposal of the lead present both in the liquid extract and in the brei after the analytical determinations is a problem the seriousness of which has reached worrying levels. The replacement of lead acetate with aluminum salts does not seem to be universally well accepted, due to problems related to the filterability, the colour of the solution and the polarimetric reading. Moreover, determination of the -amino nitrogen content must be carried out via fluorimetry when aluminium salts are used.

2) the "history" of the root cannot be given by the numerical value of the polarization alone; as far as the history of the roots is concerned we refer to beets stored for variable periods of time under unfavourable climatic conditions such as those in the Mediterranean countries. Thus, a high polarization value may be considered as an indication of good quality if the beets have only just been harvested. On the other hand if the roots have dehydrated due to storage, their polarization might be high but it does not automatically mean that they are of good quality.

It is well known that, industrially, beet quality is assessed by determining polarization as well as some other parameters, in a cold brei extract, obtained using lead or aluminum salts as mentioned above. The other parameters are sodium and potassium, which are determined by flame photometry, -amino nitrogen determined by colorimetry or fluorimetry and, recently, glucose and fructose which are determined enzymatically. The whole analytical system is automated and the determinations are reproducible and rapid, in particular as regards the first three parameters mentioned above. All of these data are used via computer for the prediction of some fundamental technological figures using suitable formulae. In particular, such formulae should forecast the purity of thick juice, the amount of crystallizable sugar and the amount of sugar remaining in the molasses, all of them obtainable by processing a single beet sample. The criticisms traditionally raised against this evaluation system are as follows:

- the cold extraction of the brei is carried out utilizing lead or aluminum salts which give the problems mentioned above;
- there are many formulae, each obtained under different environmental conditions;
- such formulae are continuously modified due to the fact that, even in the same geographical area, the environmental and pedological conditions change, i.e. as a result of modifying traditional fertilization criteria;
- in the case of beet diseases, as well as in the case of roots stored under unfavourable conditions for variable lengths of time, the formulae are often unreliable;
- the well known parameter termed the "alkalinity coefficient" often does not give a sufficiently reliable evaluation of the real alkalinity of the factory juices so resulting in necessary additions of alkali or acid in the process;
- the content of soluble amino acids in the cold extract cannot be directly related to the amount of nitrogenous substances which are extracted at the high temperature and the long time of contact between aqueous solutions and cosettes in the diffusers;
- the evaluation of the nitrogen content in the cold extract is carried out with respect to a single amino acid although, in general, it is the major one present in the extract;
- the brei is obtained by cutting a certain number of roots with a multiple saw, so causing the breakage of a great number of cells and these transfer their whole contents to the extraction solutions;
- the thick juice purity data obtained using the formulae are often markedly higher than those observed in the factory, although we must bear in mind that, not only are the roots processed, but also their crowns and, very often, even petioles and leaves which undoubtedly decrease the factory juice purities.

As far as beet seed breeding is concerned, the various varieties are marketed utilizing the available agronomical data and the figures for the estimated

technological value obtained by assuming the formulae mentioned above are reliable.

THE NEW PROPOSAL

The new proposal presented here does not involve a modification of the general criteria followed up to now in beet seed breeding, but is based on the assumption that such criteria must be related to methods which are more suitable for the evaluation of both agronomical and industrial qualities.

Bearing in mind the points mentioned above, we have tried to find criteria which are less complicated, more generally applicable, environmentally compatible and possibly more objective for the evaluation of the beet quality, starting from the following concepts:

- as well as the determination of the beet sugar content obtained without using reactants containing lead or aluminum salts, it is also necessary to determine the dry matter content. This analysis is absolutely necessary to confirm that high sucrose concentrations are not caused by dehydration of the roots. Eventually, some other important parameters can be determined, such as the total nitrogen and marc contents.
- it is necessary to prepare hot extraction juice samples following criteria which are as close as possible to the conditions utilized in obtaining factory raw juices. The purity quotient, as well some other important parameter i.e. the nitrogen content of such a juice, have to be determined using rapid, automated, sufficiently reliable and non-polluting methods. In this connection then it is necessary to bear in mind that, if a factory raw juice is of high purity, then it is highly probable that the corresponding thick juice will be of high purity and the crystallizable sugar yield will be high whilst the amount of sugar in molasses will be low.

In order to reach the above goals we had first to design a machine which could simulate the processes occurring in factory diffusion plants. Such a machine, designed and built, at the moment as a prototype, by the RE.LO.BO. Company (Figure 1) was tested during the 1994 campaign (18). It was able:

- to continuously mix with a suitable amount of water a certain amount of brei coming from the normal sawing device;
- to rapidly heat the mixture to the same temperature as in factory diffusers;
- to keep the mixture stirred at fixed temperatures for a period of time comparable with the retention time in factory diffusers;
- to rapidly separate the hot extraction juice via filtration under pressure.

The juices so obtained had visual and organoleptic characteristics comparable with factory raw juices, but they had lower purities due to the excessive breakage of the brei cells the contents of which entered the digestion juice. Moreover, the analysis of the latter, carried out using an Infrazyzer 450 manufactured by Bran+Luebbe Company, was slowed down and the number of samples analyzed per hour was limited because of the lengthy reading time. Therefore, some points had to be cleared:

- a) the setting up of a device for the preparation of the micro-cossettes able to be used in the RE.LO.BO. system;
- b) the comparison of the quality evaluation data between the traditional and the new proposed systems;
- c) the evaluation of the possibility of utilizing a NIR analyzer able to give results rapidly;
- d) to judge whether the errors made using the NIR spectrometer for the measurement of Brix and polarization of the juice could be limited sufficiently to allow a reliable evaluation of the purity quotients and consequently of the beet quality.

In order to answer point a) a simple system was set up (Figure 2) which, utilizing the same types of knives as those used in factory slicers, made it possible to obtain micro-cossettes as shown in Figure 3. The latter are thinner and shorter than factory cossettes but the number of broken cells are negligible when compared with brei and, moreover, they can be easily mixed with water inside the containers of the RE.LO.BO. system (Figure 4). The latter was partially modified relative to the

previous campaign; in particular, the conical filtering vessels were replaced by cylinders on the bottom of which the filter papers could adhere perfectly without any possibility of breakage (Figure 5).

Concerning the comparison between the newly proposed and the traditional beet quality evaluation systems (point b above), 55 beet samples were analysed utilizing the traditional criteria of the Venema system and using the Wieninger & Kubadinow formula (19) for the determination of the theoretical purity quotient of thick juice. The pieces of each sample of beet leaving the traditional saw at the end of the washing step and partially utilized for the brei preparation, were used for the preparation of the micro-cossettes. In turn these were employed for the preparation of the hot extraction juice as described above. The purity quotients and the total organic nitrogen contents were determined in each sample of juice via traditional polarimetry and refractometry, and the Kjeldhal method, respectively. Figure 6 shows the comparison between the experimentally determined purity quotient of the hot extraction juice and the hypothetical purity quotient of thick juice calculated using the Wieninger & Kubadinow formula. We can see that the correlation coefficient is rather low (about 0.7); it means that the two criteria for evaluation of the characteristics of the beet samples are not perfectly superimposable, which on the other hand was to be expected. Even the comparison between the α -amino nitrogen content determined via the Venema system (as meq/g beet) and the total organic nitrogen content determined on the hot extraction juice (as g % dry matter), shown in Figure 7, gives a rather poor correlation (0.47). This confirms that the traditional evaluation of the α -amino nitrogen content in the beet samples does not necessarily correspond with the amount of nitrogen which can be found in the factory juices. A little higher correlation coefficient (0.76) is shown for the comparison between the purity quotients and the total organic nitrogen contents in the hot extraction juices (Figure 8). However, we believe that such a correlation cannot be sufficient to unequivocally claim that a juice having a low nitrogen content will have a high purity quotient. Bearing in mind the problems arising from the presence of nitrogen compounds in sugar processing especially regarding juice coloration, we judge that as well purity quotient, determination of the total organic nitrogen content must be carried out in order to determine the beet technological value.

On the other hand, it is obvious that both the purity quotient and the total organic nitrogen content cannot be routinely assessed by traditional methods on the large number of beet samples which are analyzed daily in a breeding laboratory.

Therefore we consider that the only technique which can make it possible to achieve such a task is the NIR technique which has recently been widely applied in the sugar area for the control of many parameters. Such parameters include both solid (beet, cane, sugar, sludges, etc.) and liquid products using both off- and on-line machines (2-17), and also apply to sugarcane breeding (1).

We have already used such a technique satisfactorily in the past for the measurement of dry matter, polarization and the total organic nitrogen content of beet factory raw juices (16, 17). Preliminary experiments carried out in the 1994 campaign (18) on hot extraction juices from beet brei samples using an Infraalyzer 450 with 19 filters established that the relevant readings caused a decrease in the number of samples per hour analyzed. In fact, the reading time was about 45 seconds plus the time for rinsing the cell. The new type of spectrometer used in the 1995 campaign (an Infraprobe which utilizes an interferometric laser system) is capable of carrying out one reading per second. Therefore, this machine is able to analyze that number of samples per hour which is comparable with the number normally analyzed in the tare laboratories, while allowing a rinsing time of 10-15 seconds and providing 10-15 readings on the same sample, so obtaining more reliable average data.

It was obviously necessary to verify if, using this rapid type of instrument, the data obtained would have a reliability at least equal to that cited in the literature, so that it could be utilized for beet seed breeding. Moreover, new calibration curves had to be prepared for the new instrument; at the moment we have only measured Brix and polarization of the juices.

Figures 9 and 10 show the NIR calibration data for Brix and polarization of hot extraction juices. In both cases, correlations between laboratory and NIR data are optimal, showing values higher than 0.99; even the standard errors can be considered satisfactory with dry matter values being a little less than 0.1 and polarization values being a little higher than 0.1.

The most interesting figure for evaluation of the characteristics of the hot extraction juice, and consequently of the beet quality, is the value of the purity quotient given by the ratio between polarization and Brix multiplied by 100. Obviously, even though the standard errors of the dry matter and polarization figures are relatively low, when we calculate their ratio expressed as a percentage, the standard error increases. However, we believe that, as shown in Figure 11, a

correlation coefficient of greater than 0.91 and a standard error less than 0.9 can be well accepted because it fits with the data given by other authors for raw juice (8). As an example, if the figures are to be considered for breeding beet seed, apart the agronomic parameters and the nitrogen content of the juice, we can suppose obtaining an average value of 83 for the purity quotient of a series of samples in a single series of tests. Then, we can statistically assume that all the samples having purity quotients higher than 84 have good quality characteristics whilst samples showing purity quotients of less than 82 have poor characteristics and hence have to be discarded. Of course, samples having purity quotients of between 82 and 84 would be taken into account for further investigations.

CONCLUSIONS

The experiments carried out in 1995 made it possible to finalize the method details which, in our opinion, are indispensable for breeders who decide to utilize this new criterion for the evaluation of beet technological quality.

The proposal made previously (18) and set out in Figure 12 can now be accepted bearing in mind that:

- it is possible to prepare the hot extraction juice from the numerous beet samples to be analysed in the tare-laboratory, using a system like such as that of the RE.LO.BO. Company and utilizing microcossettes;
- use of the NIR spectrometer makes it possible to obtain all the necessary analytical data quickly enough for both solid samples and hot extraction juices.

We believe that the new beet quality evaluation criterion can be considered by the breeders because it presents the following advantages:

- it evaluates those characteristics of juices which can be compared with the factory raw juices;
- it makes it possible to evaluate the beet characteristics independently of the application of different formulae, the defects of which have been reviewed above;

- it eliminates problems associated with the use of polluting reagents in the determination of polarization;
- it gives data which we consider important and which up to now were not routinely obtained, such as the beet dry substance and the total organic nitrogen content of the extraction juice;
- the preparation of samples and the necessary analyse can be carried out as rapidly as in the traditional system.

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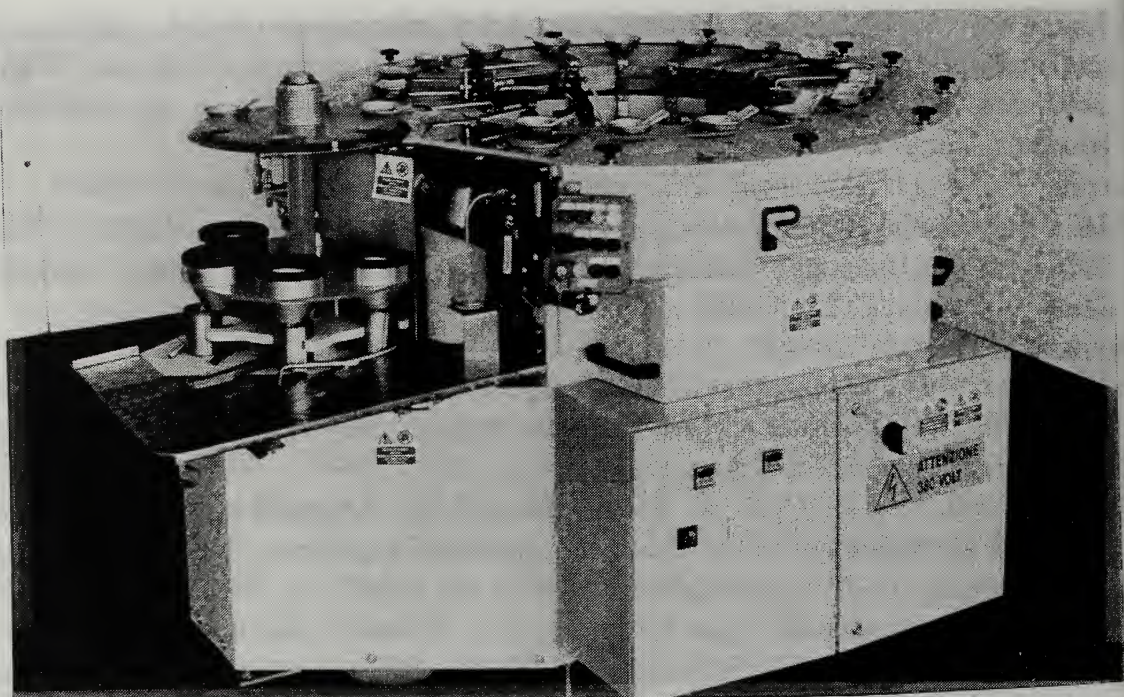


Figure 1. View of the whole extraction apparatus.



Figure 2. Interior of the device for preparation of the micro-cossettes.



Figure 3. Micro-cossettes.

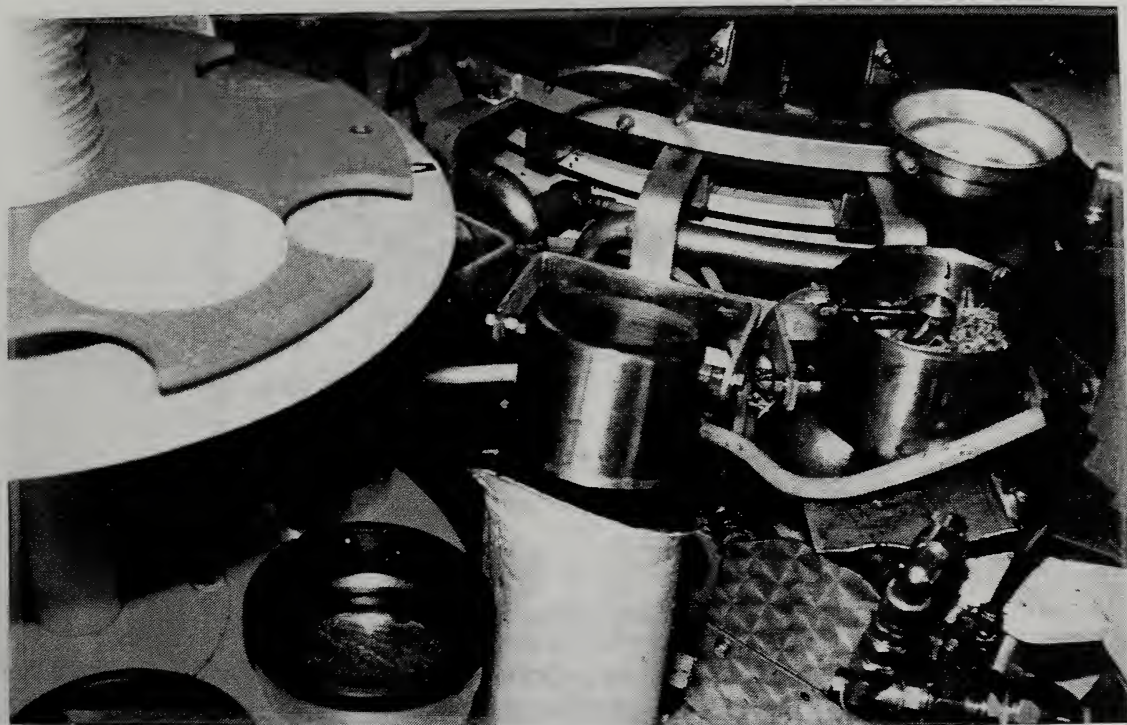


Figure 4. Micro-cossettes inside one of the vessels of the extraction apparatus.



Figure 5. Detail of the filtration system.

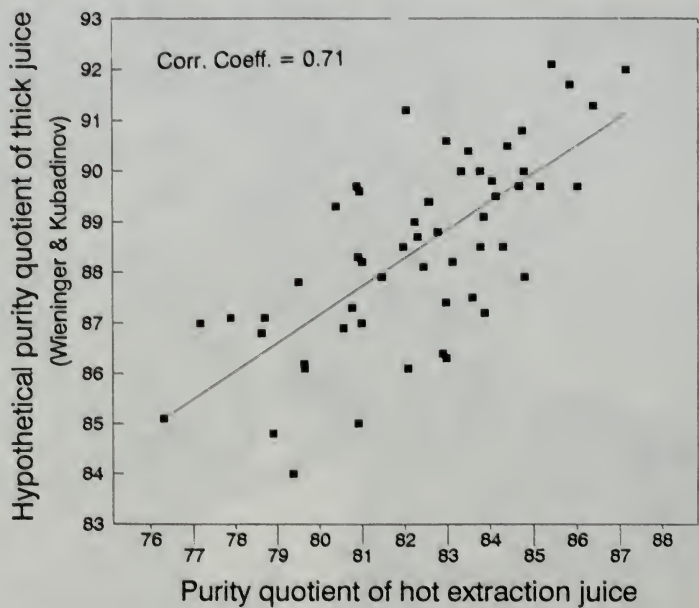


Figure 6. Comparison between the experimentally obtained purity quotients of the extraction juices and the hypothetical quotients of the thick juices calculated via the Wieninger & Kubadinow formula.

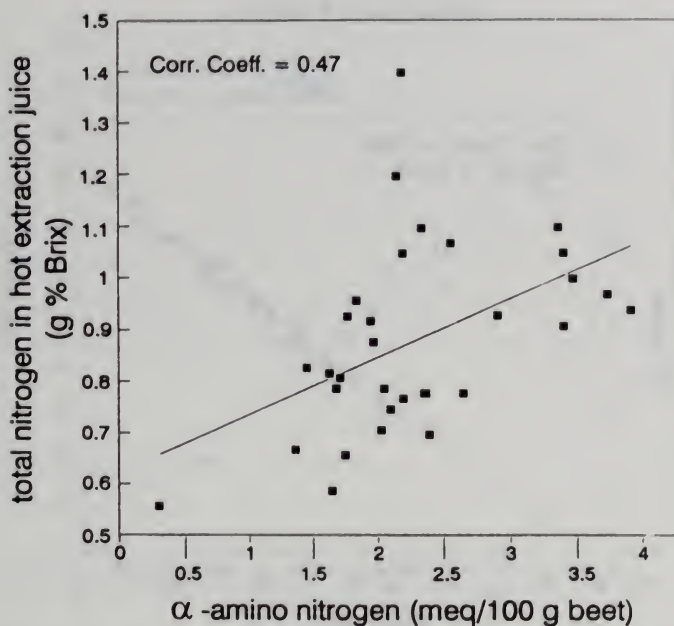


Figure 7. Comparison between the α -amino nitrogen content determined via the Venema system and the total organic nitrogen determined on the hot extraction juice.

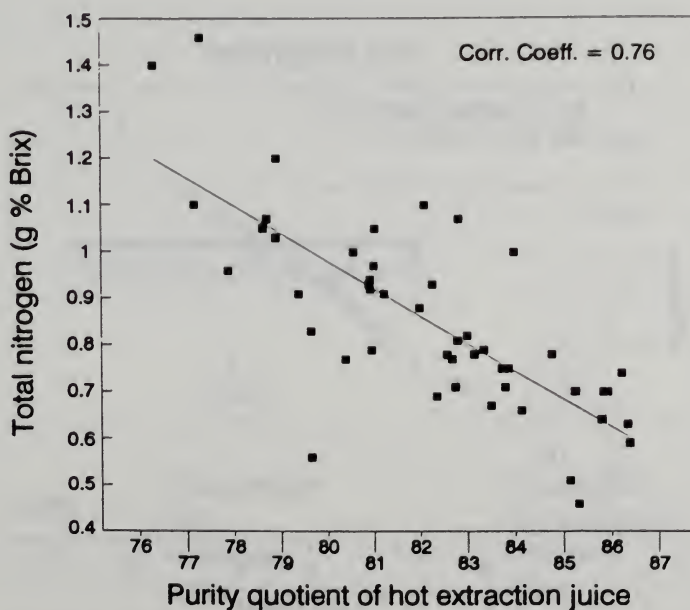


Figure 8. Comparison between purity quotients and total organic nitrogen contents of hot extraction juices.

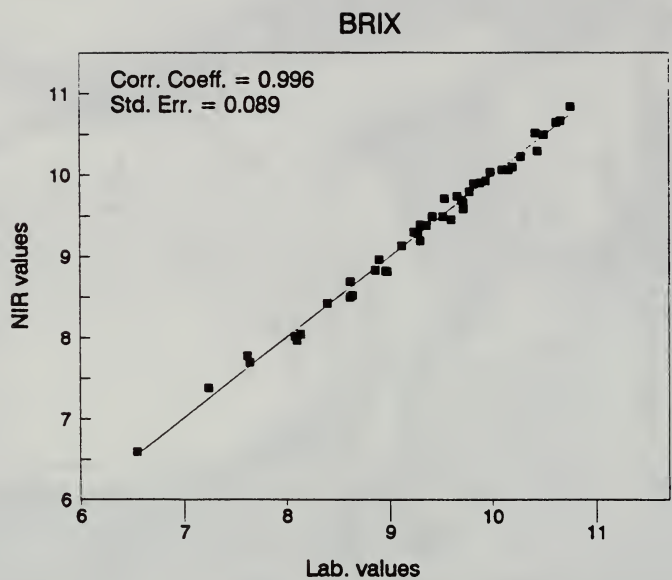


Figure 9. Calibration curve of the NIR spectrometer values against the dry matter contents (Brix) of the hot extraction juices.

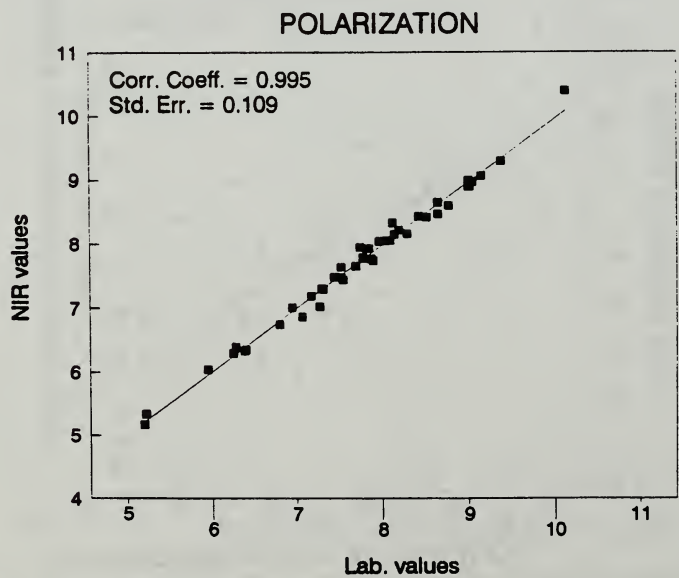


Figure 10. Calibration curve of the NIR spectrometer values against the polarization readings (lab. values) of the hot extraction juices.

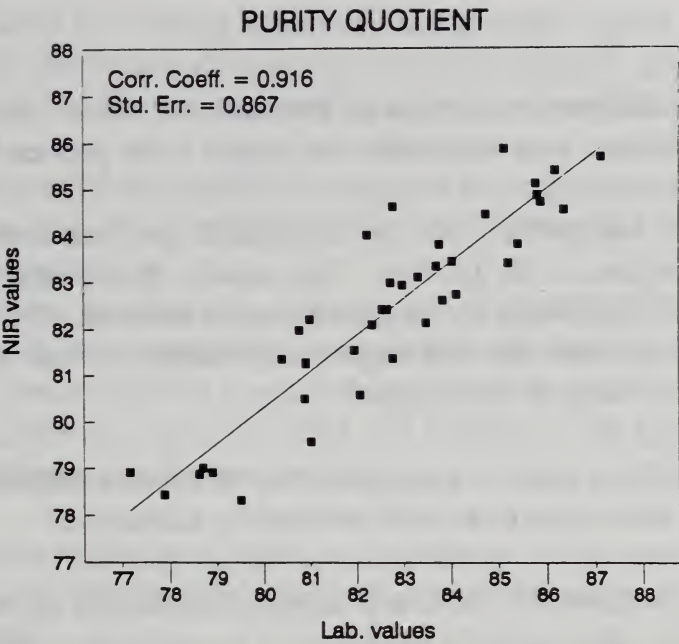


Figure 11. Comparison between the purity quotient values of hot extraction juices obtained in laboratory and calculated from the NIR values.

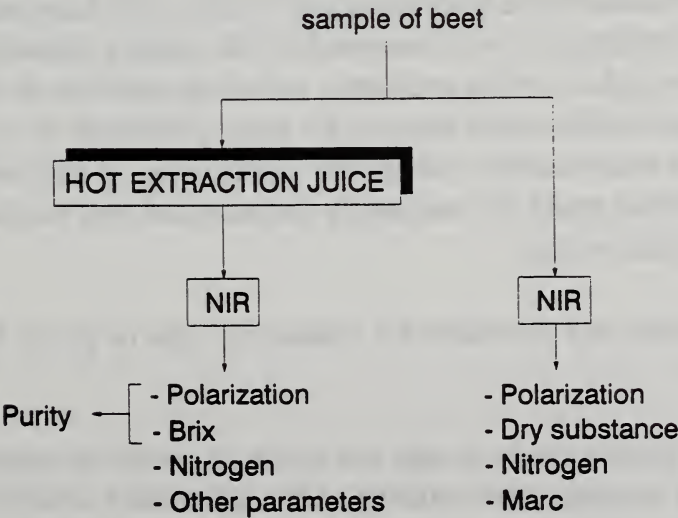


Figure 12. Proposal for brei analysis by using the NIR technique together with hot extraction juice preparation.

DISCUSSION

Question: Should we be concerned because the purity does not distinguish between removable and non-removable non-sugars in the process?

Vaccari: Yes, the purity does not distinguish between removable and non-removable non-sugars in the process. But usually in any one sugar factory the amount and type of removable non-sugars are quite constant. On the other hand, at least within limited areas, the non-sugar composition, as well as the purification coefficient do not change dramatically.

Question: You have made a change in your extraction methods, from 1994 to 1995. Have you now come to an ideal method for extraction?

Vaccari: The machine we use is a prototype, obviously, so we make changes. We think that some changes could be realized if we scale up to industrial scale. We would need a better control of the inner temperature. There are problems with discharge of solution and microcossettes in the funnel. This should be better automatized.

Question: Are you working with beet seed breeders about this approach?

Vaccari: Yes, because these trials have been made in the laboratories of the seed breeders. Seed breeders are very interested in this system, because they think that the new system can solve various problems, including pollution problems.

Breeders cannot decide which formula for sugar prediction is correct; each year various companies and countries change the coefficients, or add new factors.

So if this method could be completely automatized and verified, the breeders would find it very interesting.

Question: Is there any provision for measuring tops or green material? Could that be included?

Vaccari: If you want to include tops and leaves, it would be necessary to prepare cossettes without topping, after washing. Obviously there would be a decrease in purity. It would be more similar to the sugar factory, where tops and leaves are not removed. They contain a lot of non-sugars. If we prepare cossettes with whole beet, we will obtain data that is more reliable in comparison with the sugar factory.

Question: Thanks for relating agronomy and process - good to see that. This question may be premature: do you have any idea of costs at this point? What would be capital expenditure for a breeding company to convert to this system?

Vaccari: The cost of the machine to prepare hot extraction juice is difficult to evaluate because this is a prototype. The cost of the NIR varies. There are different types of apparatus.

The extraction apparatus is on the order of \$150,000, for a factory scale machine. The NIR instrument can vary from \$30,000 to \$100,000. The NIR we have employed uses a cell of 0.5 mm pathlength. In my opinion, in future we will use probes, not cells that must be filled and emptied. We hope to use a probe apparatus from Perstorp in the next campaign.

Question: On the reliability of purity measurements: if you were to split one beet in half, and run each half separately, how close would those purity numbers be, and how reproducible?

Vaccari: We analyzed subsamples from the same cossettes. The error is within laboratory error.

We have also tried using different sizes of samples of cossettes, and changing the solids: water ratio. We obtain practically the same results with different sample sizes. The difference among different dilutions was about 0.1-0.15 units.

THE EFFECT OF SOME POLYSACCHARIDIC NON-SUGARS ON THE RATE OF SUCROSE CRYSTAL GROWTH

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ABSTRACT

The effect of dextran, arabinogalactan and high molecular weight ($MW > 10\,000$) fraction of second runoffs and thin juices on rate of sucrose crystal growth was studied using two experimental techniques. These techniques are the end-to-end method which consists in rotating sample tubes containing supersaturated solution and a limited number of crystals (~ 20) and another method designed in the laboratory which consists in continuously feeding 100 g of massecuite (50 % crystals) with saturated solution brought to a supersaturation $\sigma = 1.10$ by cooling.

The temperature was 30°C for both methods. A first step consisted in determining the rate of growth in pure sucrose solutions which was compared to the data quoted in the literature. The amount of impurities (NS) was such that $NS/W = 0.50$ and the supersaturation $\sigma = 1.10$. Three grain sizes were experimented A.M. ~ 0.50 ; 0.80 and 1.52 mm.

The rate of growth in pure solution was about $734\text{ mg.m}^{-2}\text{ min}^{-1}$ for the end-to-end method and $938\text{ mg.m}^{-2}\text{ min}^{-1}$ for the massecuite method which is comparable to Kukhareenko's results. This denotes a synergistic effect in massecuites already mentioned by A. VanHook (ISSCT, 1977). The rate of growth is decreased in presence of pure polysaccharides and high molecular weight fraction of thin juice.

INTRODUCTION

The quality of crystalline sugar depends on some of the impurities concentrated in the technical solutions of sugar refinery during the process (especially in molasses, second runoffs ...). Moreover, the polysaccharides adsorbed at the surface of the crystal may have some influence on the stability of white sugar. In beet sugar refinery, polysaccharides have mainly two origins. Arabinogalactans, for example, originates from the cell wall. The microbial spoilage (*Leuconostoc mesenteroides*) and frost damage of sugarbeet tissue may originate in dextrans of various molecular

weights (14, 15). Furthermore, analysis of second runoffs from beet sugar refinery shows that the polysaccharidic fraction essentially consists in arabinogalactan. (13).

The presence of these polysaccharides at the surface of sugar crystals may affect crystal habit. Occlusion and crystal elongation along the c - axis are observed in presence of dextrans (8, 10). Together with the change in morphology of the crystals, the presence of impurities in the growth solution also modifies the solution characteristics, especially viscosity. It affects other physico - chemical properties like solubility, hydration and the rate of crystallisation. In order to improve the quality of sugar, we have chosen to study the influence of polysaccharides on various factors affecting the production of crystallised sucrose. A previous paper, presented at the CITS meeting in Munich (9), dealt with the solubility of sucrose in pure solution and in presence of non - sugars. Analysis of viscosity, thermal behavior and F.T.I.R. spectra of sucrose solutions with or without impurities showed us that some interactions between sucrose and polysaccharides may occur via water molecules.

To approach the complex mechanism of crystallisation, it is necessary to determine accurately the solubility of sucrose, which is the major parameter in the different steps of this mechanism.

We now present results obtained in our laboratory using two experimental methods. The first one reproduces the conditions of massecuite and the second applies the single crystal technique by means of an end-to-end crystallisation system.

PREVIOUS WORK

Determination of the rate of growth proves to be one of the most important laboratory means of approaching the study of crystallisation mechanisms. The first relevant work in the field goes back to 1910 - 1930. Kukharenko, in 1922, (7) used some flasks hermetically sealed, fixed to a vertical disk and immersed in a thermostat. The set can rotate at 10 - 15 r.p.m.. Crystals were weighted before and after crystallisation and were considered as spheres. Using an average coefficient $k = S / p^{2/3} = 4.12$, Kukharenko determined a crystallisation rate expressed in $\text{mg.m}^2 \text{min}^{-1}$.

VanHook et al (12) marked the forties - sixties while giving a more theoretical interpretation to experimental results. With a simple apparatus, he extended the work of Kukhareno to temperatures as high as 100°C and concluded that, below about 50°C, transport phenomena are not essential factors in determining growth rates.

Devillers (4) worked on crystals with large dimensions, fastened to a sting and disposed in a syrup flow with adjustable velocities and temperatures, as shown in Figure 1. He also noted that crystallisation rate increased with supersaturation and studied the influence of impurities on the rate and the habit of crystals.

Gerasimenko and Golovine (12) have used an apparatus similar to that of Kukhareno for moderated temperatures, between 20 and 60°C. For higher temperatures they directly weighed the crystal in the crystalliser. The crystal was suspended to a quartz fibre balance. They, also, measured the viscosity and the refractive index. In fact, growth rate measurements can be made either on single crystals or on a population of crystals.

METHODS AND MATERIALS

End-to-end crystallisation

Vaccari et al (11) used the end-to-end crystallisation method to study the morphology of crystals which grow in presence of oligosaccharides or other kinds of impurities.

In our case, this method, illustrated in Figure 2, was used to obtain velocity of crystallisation of sugar in solutions without or with non - sugars. Some tubes are filled with supersaturated solutions with eventually some impurities at the ratio non - sugar / water of 0.5 . These tubes can rotate (at 6 r.p.m.) in a thermostatic bath at the crystallisation temperature 30°C. Each tube contains some crystals (at the most 20) homogeneous in size and shape. Each crystal can float freely in the supersaturated solution. Growth solutions used for each test are fresh to keep supersaturation constant. After crystallisation, crystals are placed in a saturated solution at room temperature and we determine the crystal dimensions, before and after crystallisation, along the b and c-axis under microscope for the 0.5 and 0.8 mm crystals; for the larger crystals (1.52mm) we measure the a, b and c dimensions with a calliper square.

Main dimensions a , b and c give an average rate expressed by the dimension of length / time. In order to express it in weight / area * time, we have to use a shape factor. Bubnik and Kadlec (2), propose in the case of sucrose crystal the shape factor 0.711. The sucrose crystal shape is supposed to have a simple geometric body like a parallelepiped with main lengths L_b and L_c . As they determine this shape factor in pure solutions, we have to use it with an approximation about its validity for impure solutions.

We also have evaluated a shape factor by means of crystal sieve fraction photographs. With the observation of 100 crystals per sieve fraction, we find an average shape factor of 0.413. However, we prefer to use the Bubnik and Kadlec's shape factor, which is a statistical value.

For some tests, crystals were directly weighed in order to obtain an overall crystallisation rate expressed in $\mu\text{g}/\text{min}$. This technique of single crystal is ideal for studying the growth mechanisms. It leads to the obtaining of faces growth rates which can hardly be correlated with an overall growth rate.

Massecuite crystallisation

To account for other crystals influence, the massecuite technique is more adapted. The apparatus, we have set in our laboratory is shown in Figure 3 and consists in a system of flasks kept at constant temperature. This system is inspired from the technique developed by Devillers (4). In our apparatus, the graining charge made of sieved crystals (50g) and a saturated syrup (50g), at 30°C ($C = 70.3\%$ DS) containing the desired quantity of non - sugars (non-sugar/water = 0.5), is regularly fed with another syrup, by means of a pump. The feed syrup is stored at 65°C in order to have at 60°C the saturation ($\sigma = 1$), with a concentration of 74.3% DS. The crystallisation flask, placed in a thermostatic bath at 30°C , is equipped with a slow agitation to keep the mass homogeneous.

We have to note that we used, for the first tests, a pump with a rate of flow of 3500 g / h . As this pump worked discontinuously, we have to calculate an average rate of crystallisation taking into account the feed of syrup time. The crystallisation is controlled by measuring the temperature and by refractometric analysis of the mother liquor. The refractometric Brix is a direct measurement of the dry substance ($\text{DS}\% = \text{Brix}$). From this dry substance, the quantity of sugar dissolved in the mother liquor can be deduced ($\text{DS} - \text{non sugar}$).

The surface of 100 g of crystals can be estimated in first approximation by:

$$S = 0.4 / M.A.$$

where M.A. is the mean aperture.

Knowing the crystal weight in the massecuite ($CR_{(i)}$ = total sugar in the massecuite - sugar in the mother liquor), the crystallisation rate V_c is easily calculated in the time period $\theta_{(i)} - \theta_{(i-1)}$

$$V_c = CR_{(i)} - CR_{(i-1)} / [S_{(i-1)} \cdot 60] \cdot [\theta_{(i)} - \theta_{(i-1)}]$$

A computer programming of all these calculations allows us to follow the crystallisation rate only by introducing the values of refractometric Brix and temperature.

Materials

With these two methods, adopted in our laboratory, we have studied the influence of the crystal size upon the crystallisation rate, and also the influence of some impurities.

We used, for our studies, sugar with three different sizes (M.A. = 0.5 ; 0.8 and 1.52 mm) supplied by Compagnie Française de Sucrierie (Bucy). The pure polysaccharides used are Sigma products : Arabinogalactan from larch wood (A2012 purity > 98%) and dextran produced by *Leuconostoc mesenteroides*, Strain n° B-512 M.W. = 73000 (Sigma D-1390). The high molecular weight (MW > 10 000) fraction of second runoffs and purified sugarbeet thin juices were obtained by ultra-filtration with the collaboration of Générale Sucrière, Sucrierie de Guignicourt and Sartorius France.

RESULTS AND DISCUSSION

Massecuite results

The literature on the crystallisation rates for tests of massecuite at low temperature like 30°C is rather scarce. The software realised at laboratory allows calculation of

the crystallisation rate for the tested supersaturation and for the supersaturation $\sigma = 1.1$.

An example of simulation is shown in Table 1. This calculation illustrates the case of the system working with a constant pump flow of 180 g/h, at 30°C. We obtain, for a supersaturation $\sigma = 1.1$, an average crystallisation rate of 60 g/m².h. This kind of test of simulation allows determination of the best conditions to lead as better as possible the massecuite crystallisation.

First of all, we test three sizes of seed crystals, in pure solution. Data reported in Table 2 show that the crystallisation rate increases with the crystal size. In our conditions of work, there is a linear dependence between the crystallisation rate and the mean aperture. These results are in agreement with the explanation given by Guimaraès et al. (6).

These authors have studied the effect of crystal size at 40°C using a fluidized-bed crystallisation. They note that the overall growth rates increase with supersaturation and the crystal size. For these authors and also for Garside et al (5), the mechanism of growth size depend on the volume diffusion of solute to the crystal surface, the crystal size effect on the equilibrium solubility (the Gibbs-Thompson effect) and finally size dependent surface integration kinetics.

On the whole, the total growth rate can be considered as the sequence of two main processes. The first, the diffusion or mass transfer, consists in the transfer of molecules from the bulk solution to the crystal surface. The second one involves the insertion of molecules at the surface of the crystal (the surface reaction process). It is well known that, when the growth temperature is low, the surface reaction controls the crystallisation. By contrast, the volume diffusion prevails at high temperature. Dedek (3) observed that, for a fixed degree of supersaturation of 1.05 and at the temperature of 30°C, the crystallisation rate of sucrose compared with the theoretical diffusion rate is equal to

$$\text{Diffusion rate} / \text{Growth rate} = 4.5$$

This result would suggest that at low temperature, the crystallisation process is not diffusion controlled. Dedek also indicated that excess sucrose is available at the growing crystal face at temperatures lower than about 45°C. Moreover, in a crystalliser, where crystals are maintained in suspension by agitation, the large

crystals grow faster than the small ones, because of a higher relative solute / solution velocity.

End-to-end results

The single crystal technique using rotating tubes allows determination of the crystallisation rate of different sizes of crystals. Figure 4 represents the evolution of crystallisation rate (expressed in $\mu\text{g}/\text{min}$) as a function of supersaturation, for two different mean apertures. We note that, at the same supersaturation, the larger the crystals the higher the growth rate. The obtained curves present, as expected, various aspects depending on the increasing supersaturation. Three zones can be distinguished: a parabolic, a linear and a logarithmic one. For the M.A. of 1.52 mm crystals, the growth rate follows the parabolic law up to a supersaturation of ~ 1.03 , then the curve has a linear pace for supersaturation below 1.15. Beyond $\sigma \sim 1.20$, the growth rate has a logarithmic dependence with the supersaturation.

This kind of curve shapes is correlated, according to Aquilano et al (1), with the different consecutive mechanisms of crystallisation. Indeed, at low supersaturation the spiral growth mechanism dominates and the relationship (R, σ) obeys to a parabolic law. For this first zone, the rate of nucleation is limited by the transport of matter to the steps by surface diffusion. Then with the increasing supersaturation, 2D nucleation occurs between steps so that isotherm growth follows a linear pace. The final relationship (R, σ) is observed when the nucleation frequency is limited by the diffusion of growth units from the volume to the terraces.

On the following figures the crystallisation rate is obtained by the observation of crystals along various crystallographic axis and is expressed in $\text{mg}/\text{m}^2.\text{min}$. Figures 5- a, b and c represent, for each tested size of crystals, the increase of the growth rate with increasing supersaturation. For the crystals of M.A. = 0.5 and 0.8 mm (Figure 5- a and b) the observation under microscope along the b and c axis leads to the obtaining of the two rates R_b and R_c . For these two sizes of crystal, the rate along the b-axis predominates as compared to c-axis rate. As concerns crystals with M.A. = 1.52 mm, the growth rate along the three crystallographic axis is shown in Figure 5- c. For these large crystals, the c-axis or a-axis rates are less important than that of b-axis. These crystals have their growth, mainly, along b-axis. Whatever the size of crystals, the rate along b-axis, in pure solution is the more significant. These results agree with the well-known fact that, normally,

sucrose crystals grown in pure solution are more elongated along the b-axis than along the c and a-axis.

The relative growth rates R_b/R_c , R_b/R_a or R_c/R_a , are represented on Figure 6- a and b. They clearly show the variation of crystal faces growth rates with supersaturation. The Figure 6 -a represent these variation for the two sizes of crystal of 0.5 and 0.8 mm. For the smaller crystals (M.A. = 0.5 mm), the ratio R_b / R_c increases constantly up to a supersaturation of ~ 1.05 . For the M.A. = 0.8 mm crystals, the ratio decreases up to the same supersaturation $\sigma \sim 1.05$. For both these two sizes R_b / R_c becomes constant beyond the supersaturation of 1.05. ($R_b / R_c \sim 2$) With the larger crystals (M.A. = 1.52 mm), represented on the Figure 6 -b, while the ratio R_c / R_a stays constant, the other ratio R_b / R_a and R_b / R_c have various behaviours. After an increasing up to $\sigma \sim 1.05$, they progressively decrease to become constant from $\sigma \sim 1.10$. We also note that the ratios, obtained for this M.A. (1.52 mm), are higher than that obtained for the small size crystals (M.A. = 0.5 or 0.8 mm). At the supersaturation of 1.10 the ratio R_b / R_c , calculated for the M.A. of 0.5 mm, is about 2. At the same supersaturation, R_b / R_c , for the M.A. of 1.52 mm, is equal to about 70. However all these results converge on the fact that, whatever their mean aperture the crystals have not a homogeneous overall growth for each face. The geometric shape is known to change during the crystallisation process. The smaller faces gradually disappear in favour of the largest, slower-growing faces.

Effects of impurities

In addition to the above studies in pure solutions, we have investigated the effect of impurities on the growth and shape of sucrose crystals with a constant ratio non-sugar/water of 0.5. We observe, on Figures 7a and 7b, the rate of crystallisation of solutions added with dextran, arabinogalactan or with impurities coming from ultrafiltrated juices (runoffs or thin juice). The growth rates in impure solutions are compared with that of the pure sucrose solutions. As expected, the presence of non-sugars reduces the crystal growth. We observe also, like in pure solutions, a superior to one order dependence between crystallisation rate and supersaturation, whatever the kind of impurity. The presence of dextran leads to lower crystallisation rates since very low supersaturation. At $\sigma = 1.10$ the rate is more than 700 mg /m².min in pure solution and ~ 10 mg /m².min in solution added with dextran. The curve shape obtained in this impure solution is very smooth and do not reach rate values higher than 400 mg /m².min. The presence of « thin juice

impurity » (Figure 7 -b) reduces the growth rate with a similar behaviour to that with dextran. The rate value stays under 400 mg /m².min. Figure 7 -b also shows the decreasing effect of impurity coming from runoffs. The decrease of growth rate observed within solution added with arabinogalactan (Figure 7 -a) seems to be less marked by the varying supersaturation than the other impure solutions. The curve concerning « runoffs impurity », for example, shows clearly the three different zones described above. The effects of all these impurities vary while comparing the same supersaturation. And, by accounting the different zones of the curve shapes (parabolic, linear and logarithmic), we note that these various impurities may have some effects on the sucrose crystallisation rate at different supersaturations specific to these impurities. Each of them reaches, for example the linear zone, at different supersaturation (near to 1.2 for the « runoffs », 1.14 for the « thin juice »).

Impurities and habit modifications

For each type of impurities, we compare the rates along b and c-axis. Figures 8 -a and b show the crystallisation rates determined along these two crystallographic axis as a function of supersaturation. In all cases, there is an increase in the rate with the supersaturation but we also note that, in the case of solution containing dextran, the c-axis rate may be higher than the b-axis rate, which indicates that there is an elongation along the c-axis (Figure 8a).

In fact, the ratio R_b/R_c is lower than one. This result agrees with Sutherland et al (10) who reported that the presence of dextran in sugar syrups increased the viscosity and caused elongation of sucrose crystals along the c-axis. They also found that an increase in temperature enhanced c-axis elongation which was also affected by the molecular weight and structure of dextran. Oppositely, observation of Figures 8b and 8c, shows that addition of arabinogalactan or runoffs or thin juice impurities in sugar solutions leads to ratios R_b/R_c higher than one. These impurities promote the crystal elongation along b-axis.

Sucrose crystals are known to have varying morphology in presence of impurities. (11) Glucose and fructose for example lead to the characteristic D-shape. Raffinose, mainly present in sugarbeet, blocks the growth of p' faces and modifies the other faces to give a final needle shape aspect. The crystal is elongated along the b-axis.

CONCLUSION

The crystallisation process is considered as a purification process by impurity ejection. Unfortunately, these soluble impurities may affect the crystallisation and especially the crystallisation rate. The two methods of crystallisation experienced in our laboratory allowed observation of the effect of the crystal sizes on growth as well as the influence of some non-sugars. Crystallisation rate increases with supersaturation and crystal size whatever the method used. As surface integration kinetics is size dependent, it may be possible to evaluate integration rates in terms of crystal sizes, by determining dissolution rates. It would be also interesting to study the growth rate dispersion, to explain the growth rate dependence on crystal size.

However, it should be remarked that crystallisation rates obtained in a crystalliser with a population of crystals, or for the single crystal do not follow the same mathematics laws because of many parameters and especially the synergy between crystals present in the massequite. Our results obtained during end-to-end crystallisation show a decrease in crystallisation rates due to the presence of impurities which leads to habit modifications. Some impurities are physically adsorbed on the different faces of the crystal by establishing hydrogen bonds. These interactions will be studied by use of parallel physico-chemical and spectroscopic methods to elucidate the origin of rate decrease at a molecular level.

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Table 1. Simulation results for the crystallisation rate in massecuite method.

Syrup composition:

Gr. de NS % gr.W: 0

Supersat. coef.: 1

Graining charge:

Saturation T°C: 41

Measured Brix: 70.3

Feed syrup:

Saturation T°C: 60

Measured Brix: 74.3

Initial pump flow gr / h: 180

Seed crystals:

Mean aperture 0.5 0 (mm)

Crystals weight 50

Test of crystallisation rate (Simulation for $V_c = 60 \text{ g/m}^2/\text{h}$, at 30°C)

Time min.	pump flow gr/h	T°C m.c.	Brix e.m.	σ	weight Crx	dim.Crx	V_c $\sigma = 1.1$
0	180	30.0	70.30	1.10	50.00	0.5 0	
10	180	30.0	70.30	1.10	54.00	0.5 13	61
20	180	30.0	70.30	1.10	58.10	0.5 26	59
30	180	30.0	70.20	1.09	62.60	0.5 39	63
40	180	30.0	70.20	1.09	66.70	0.5 50	58
50	180	30.0	70.15	1.09	71.1	0.5 62	60
60	180	30.0	70.10	1.09	75.6	0.5 74	60

Table 2: Effect of seed size on crystallisation rate.

M.A. (mm)	V_c (mg / m ² min)
0.5	938
0.87	2062
1.52	3766

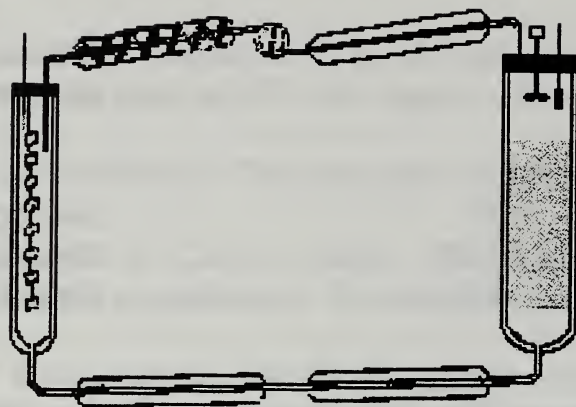


Figure 1. Apparatus used by Devillers (1967).

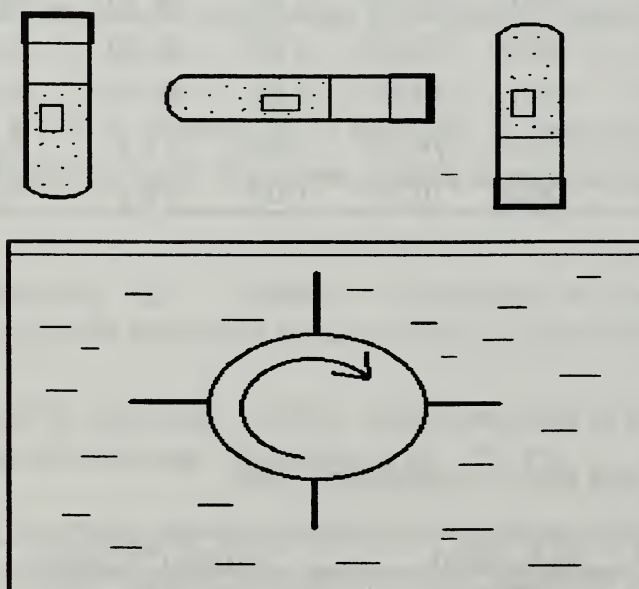


Figure 2. End-to-end crystallisation system.

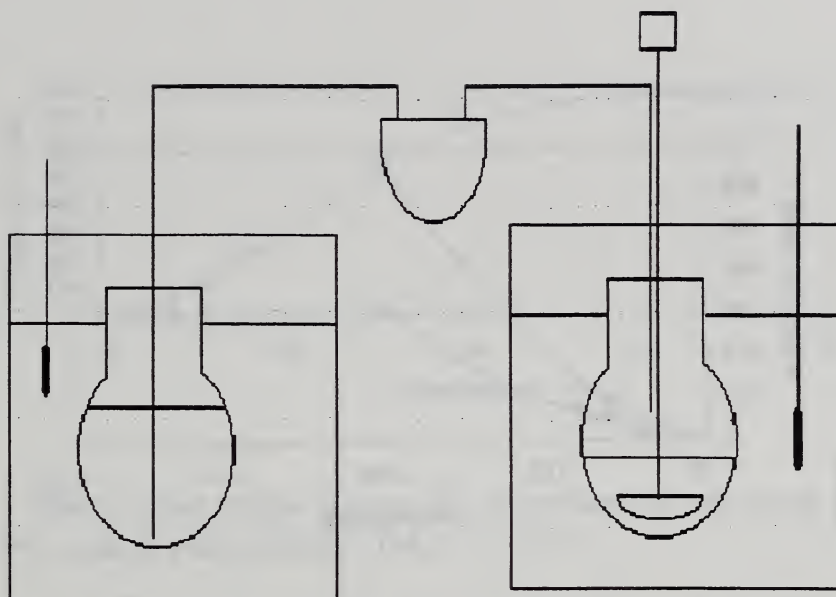


Figure 3. The massecuite crystallisation apparatus.

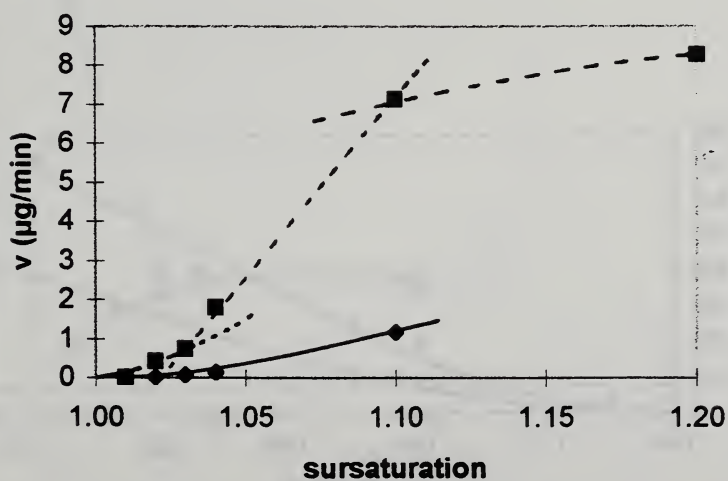


Figure 4. Effect of crystal size on the overall crystallisation rate, in pure solution. (♦): M.A. = 0.50 mm; (■): M.A. = 1.52 mm.

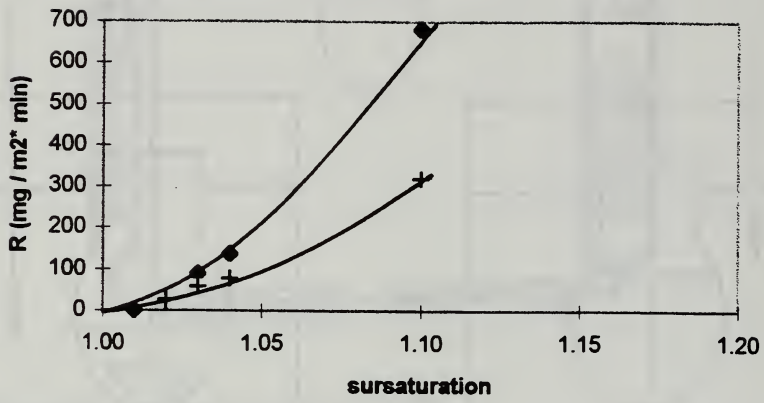


Figure 5a. Effect of crystal size on the crystallisation rate along b (\diamond) and c ($+$) - axis, in pure solution. (M.A. = 0.5 mm).

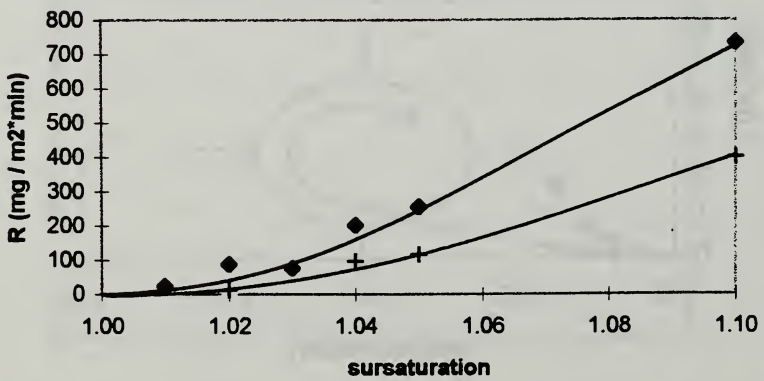


Figure 5b. Effect of crystal size on the crystallisation rate along b (\diamond) and c ($+$) - axis, in pure solution. (M.A. = 0.8).

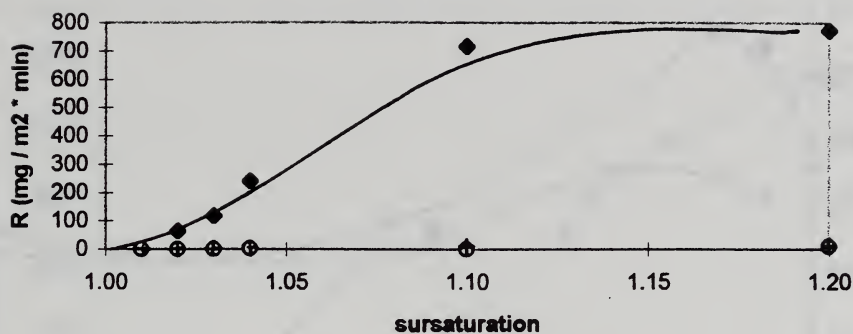


Figure 5c. Effect of the crystal size on the crystallisation rate along the a (O), b (◆) and c (+) -axis, in pure solution. (M.A. = 1.52).

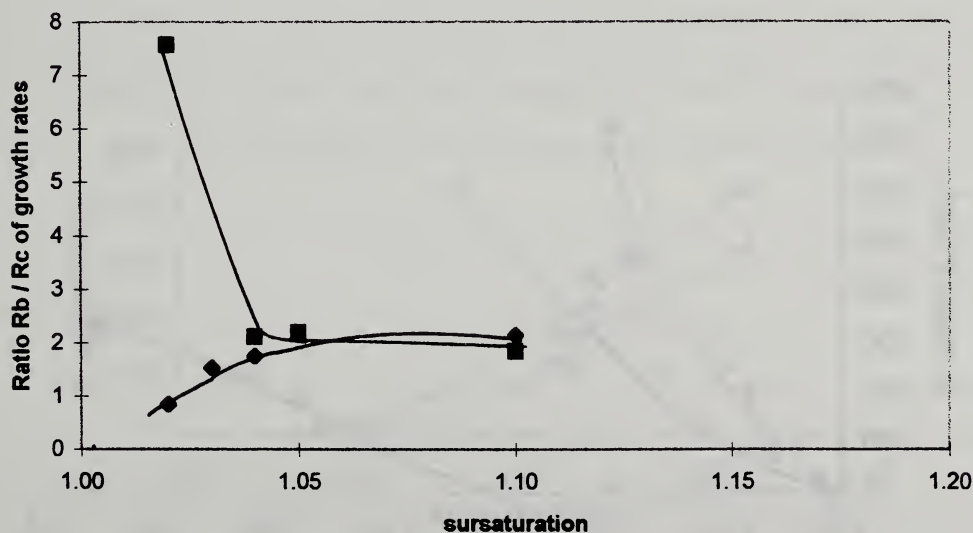


Figure 6a. Comparison of growth rates in pure solutions. (M.A. = 0.5 mm (◆) and 0.8 mm (■)).

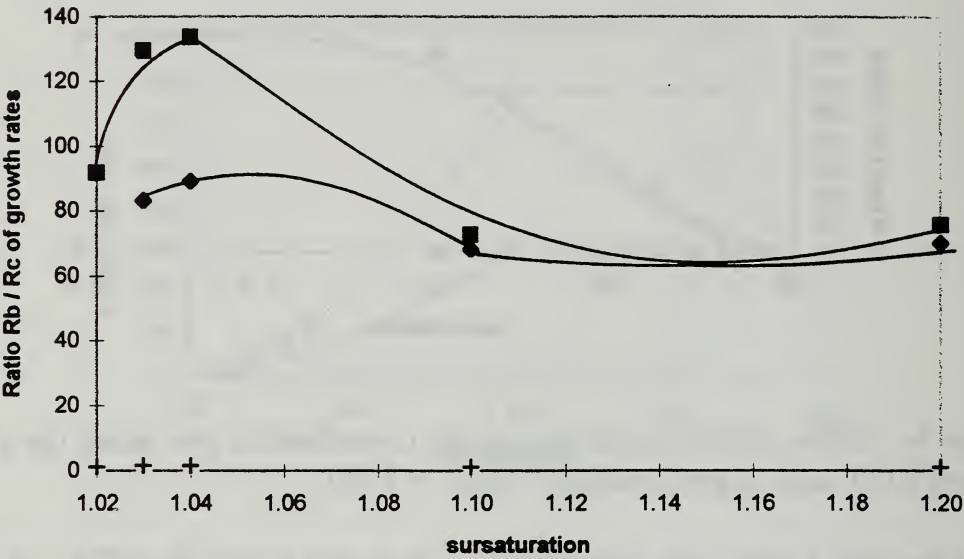


Figure 6b. Comparison of growth rates in pure solutions, R_b/R_c (◆); R_b/R_a (■); R_c/R_a (+) M.A. = 1.52 mm.

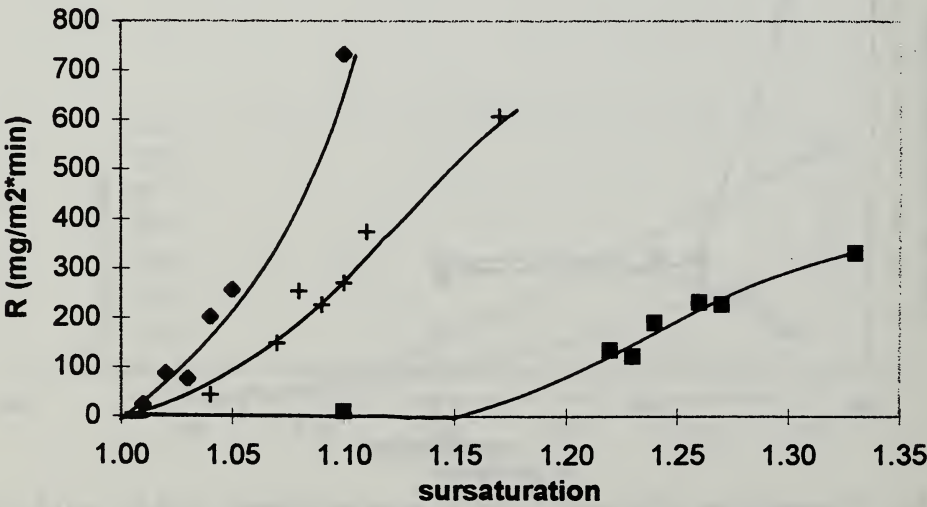


Figure 7a. Influence of impurities on growth rates, pure solution (◆), dextran (■) or arabinogalactan (+) (NS/W = 0.5).

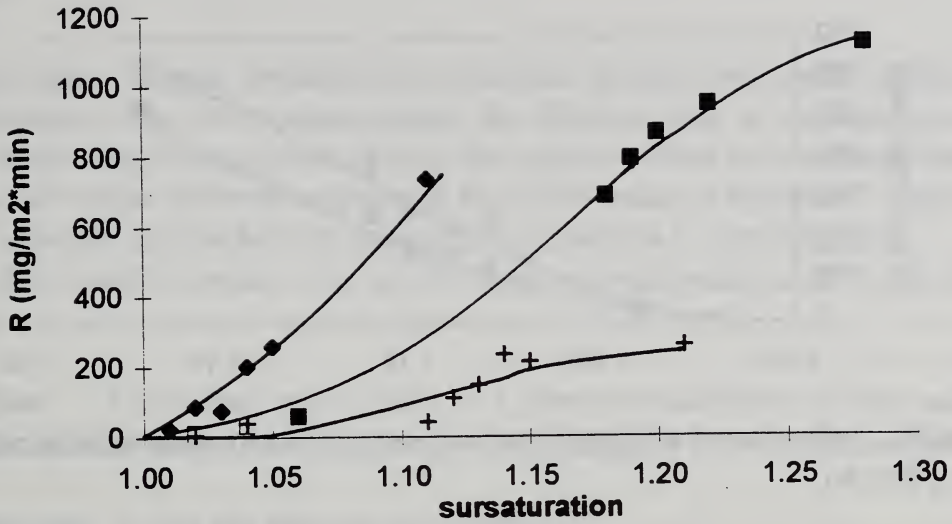


Figure 7b. Influence of impurities on growth rates, pure solution (◆), runoffs (■) or (+) thin juice (NS/W = 0.5).

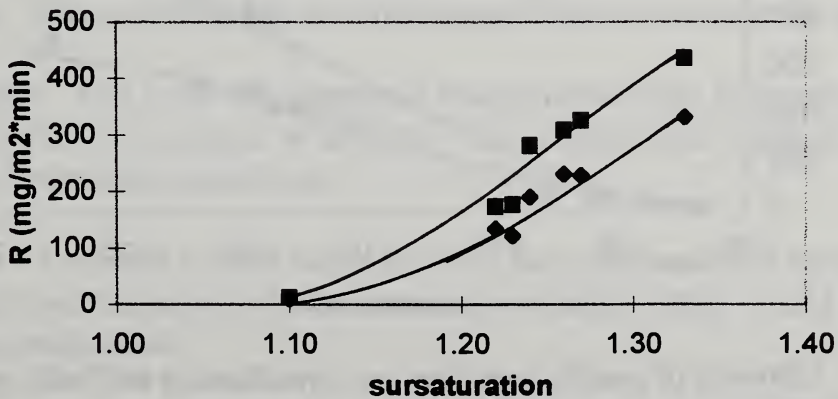


Figure 8a. Influence of dextran on crystallisation and habit modification, Rb (◆), Rc (■).

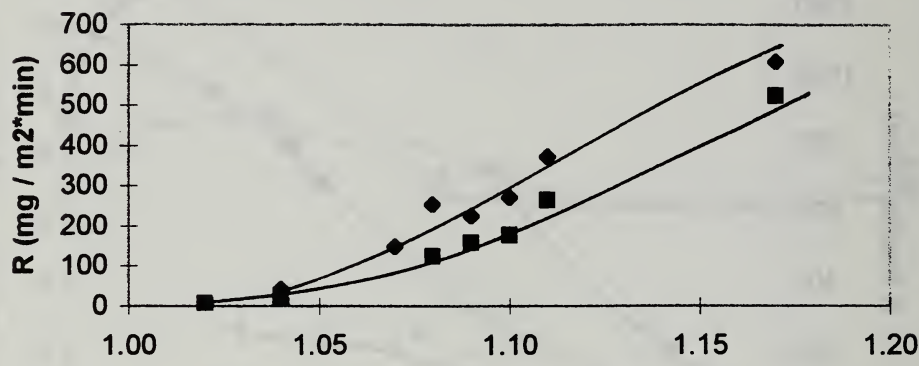


Figure 8b. Influence of arabinogalactan on crystallisation and habit modification Rb (◆), Rc (■).

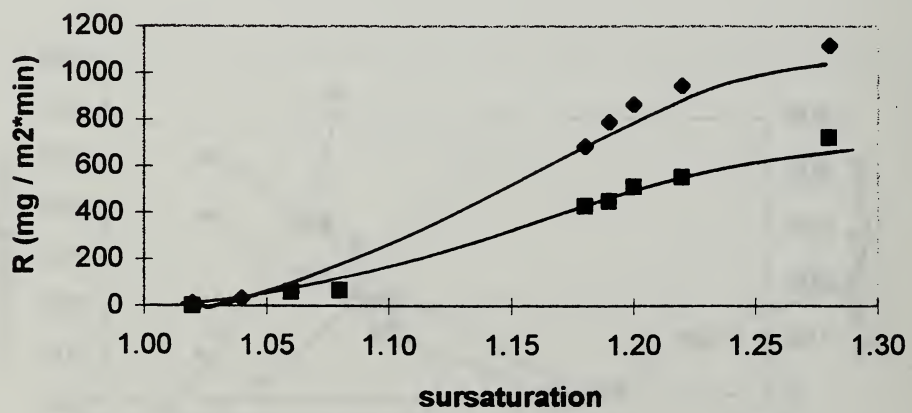


Figure 8c. Influence of runoffs impurities on crystallisation and habit modification Rb (◆), Rc (■).

DISCUSSION

Question: You showed two different mechanisms. Could you explain this further?

Mathlouthi: Sugar crystallization proceeds through two steps: diffusion and incorporation. At low concentrations, the diffusion step is not rate controlling. The crucial step is incorporation into the crystal. Prior to incorporation in the crystal, the sugar molecule must break free of its water of hydration. The way the sugar molecule approaches the surface of the crystal is a controlling step.

I did intend to make some stroboscopic measurements at the surface of the crystal.

Question: The refractometric Brix is a direct measurement of dry substance. How sensitive was the refractometer you were using for these measurements?

Mathlouthi: It was the classical, to 0.2 Brix.

Question: Normal refractometers are accurate to 0.1 or 0.2 units. You were showing differences in Table 1 of that order of magnitude.

Mathlouthi: We know the solubility curve is good as a function. In Table 1, Brix values are calculated and that is why there is more than one digit after the decimal.

Question: How much change in refractometer Brix do you normally see?

Mathlouthi: This is not very important change because the important parameters are temperature and percent of crystals. The refractometer measurement is one parameter but not the crucial one.

Question: You used a shape factor of 0.711 but you measured a factor of 0.413. If you used your experimental measurement, how much change would this have on your final conclusions?

Mathlouthi: The curve does not change. The results would be lower, but we prefer to use the Bubnik coefficient because this was made on very large numbers. The tendency is to have an exponential line whatever the coefficient.

Question: What would happen with addition of dextran?

Mathlouthi: Results are similar in general shape but the values of velocities are much lower.

Question: What was the dextran level before any effect was noticed?

Mathlouthi: It is not a matter of ppm, it is the ratio of non-sugar to water totally obtained with dextran.

Question: In answer to the prior question, the levels of impurities you added are comparable to levels found in process. Another question: have you tried other polymers as well as polysaccharides?

Mathlouthi: We are now proceeding to the study of other polysaccharides.

Question: Is this the original system? Is it from an industrial laboratory?

Mathlouthi: No, it was designed in the lab and calibrated with pure solutions.

Question: It seems to me that the small crystals have a different morphology than the large crystals. The c/b ratio is different.

Mathlouthi: The ratio is different and the rates of growth should be compared on a mass basis rather than a shape basis.

EFFECT OF STARCH ON FILTERABILITY OF CARBONATATED LIQUOR

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ABSTRACT

A laboratory filterability test, based on a bench scale pressure filter, can measure filtration problems caused by suspended solids, but will not predict refinery filtration problems caused by starch. However, it has been well established that starch will interfere with carbonatation and hence does create filtration problems in a carbonatation sugar refinery. A laboratory batch carbonatation has been developed and described. This was used to study the effect of starch on the cake resistance of the carbonatated liquor. Different affined raw sugars were carbonatated under standard conditions and their cake resistances were measured for comparison.

An alpha-amylase enzyme was used to break down the starch in order to improve the filtration characteristics of the carbonatated liquor. Experiments were carried out at 68 Brix and the time taken under various conditions measured.

A relationship has been established between the filterability caused by suspended solids and the filterability of the carbonatated liquor.

INTRODUCTION

The filtration of raw sugar in a refinery is one of the key processing steps, and if this filtration becomes difficult due to the nature of the raw sugar the refinery can quickly run into capacity problems.

Filtration problems can be caused by suspended solids present in the raw sugar (1, 2, 3, 4), but they can also be caused by starch.

The effect of starch on carbonatation, leading to filtration difficulties has been studied (2, 5, 6, 7) and is believed to be because starch interferes with calcium carbonate crystal growth. Enzymes are used quite extensively in cane mills to remove starch, and this is well documented (8, 9, 10). This is usually carried out

by injecting alpha-amylase enzyme into the partially evaporated juice between two stages of the multiple effect evaporator, often at about 40 Brix. Tsang et al has studied the removal of starch with amylase enzyme at a sugar solution of 60 Brix (10).

Alpha-amylase enzyme is also used in carbonatation refineries, and the use of enzymes at Hulett's refinery in Durban has been reported in 1995 (7). Alpha-amylase is added to low Brix sweetwater returning from secondary carbonatation cake washing, and prior to the melter.

There is little recorded information on the level of starch that will cause a problem in carbonatation, or on the effectiveness of alpha-amylase in concentrated sugar solutions, as in the refinery melter. This work reports a study of the effect of starch on the filtration characteristics after laboratory carbonatation, and the effect of enzyme treatment. It compares the cake resistance with the filterability of the raw, and shows the comparative effect of suspended solids and starch on filterability.

EXPERIMENTAL

Much of the experimental work described in this paper is carried out using a laboratory scale carbonatation. The raw sugar used for the experiments needs to be laboratory affined, and the starch level analysed. The methods for these are described below.

Affination of raw sugar

Transfer 300 g raw sugar to 450 ml of saturated granulated sugar solution in a beaker. Stir for 10 minutes at 100 rpm using a six turbine/blade agitator. Centrifuge at 3000 rpm for 2 minutes in a laboratory basket centrifuge. Remove sugar from basket and dry at 90°C.

Starch measurement

Starch was measured by a colourimetric procedure, described by Plews (11). Starch is precipitated in slightly acidic alcohol solution. The precipitate is filtered off and dissolved in calcium chloride solution. A blue colour starch-iodine complex is developed and measured colourimetrically at 600 nm. The starch content is

calculated from a calibration graph using Lintner's starch as standard. (Listed in the UK in the BDH catalogue).

Laboratory batch carbonatation

A laboratory batch carbonatation was used to study the effect of starch on the cake resistance of the carbonatated liquor. The experimental apparatus is shown in Figure 1. A jacketed reaction vessel was equipped with a sintered glass gas bubbler mounted on the side close to the bottom of the vessel. The carbon dioxide used was a mixture of 12.5% CO₂ and 87.5% N₂, and the total gas flowrate was maintained at 1100 ml/min throughout the run. The gas flow was controlled by a regulator valve followed by a needle valve. A six blade stainless steel turbine stirrer was mounted in the middle just above the gas bubbler, and the stirrer was driven by a motor set at a constant speed of 300 rpm. A water condenser was mounted on the top to minimise vapour loss. A temperature probe and a pH electrode were immersed in the liquor to record temperature and pH values.

Procedure

- 1). A fixed amount of liquor, about 450 ml, is added to the reaction vessel and raised to the operating temperature with constant stirring.
- 2). A sample is withdrawn to monitor the initial starch content.
- 3). An appropriate amount of 10% milk of lime is added to give the required lime level of 0.5% on solids.
- 4). The carbon dioxide and nitrogen gas mixture is switched on to initiate the carbonatation process at exactly two minutes after the lime addition.
- 5). The carbonatation process is stopped when the pH drops to 7.4 at 70°C.
- 6). The carbonatated liquor is retained and used to determine the cake resistance.

Filterability test of affined sugar

Affined sugar filterability is measured by the standard laboratory filtration method (3) and this is described in Appendix 1. The equipment is essentially a pressure filter and is shown in Figure 2. All of the filterability values presented in this paper are the average results of two tests.

Cake resistance of carbonatated liquor

The cake resistance of the carbonatated liquor was measured by the same filtration equipment as shown in Figure 2. The method is carried out at constant pressure and constant temperature. Filtrate volumes are recorded against filtration time. A typical plot of filtration time/filtrate volume against filtrate volume gives a straight line. The cake resistance is calculated from the slope. All of the values of the cake resistance presented here are the average of two tests.

RESULTS

The effect of starch on cake resistance

Three different sources of raw sugar were chosen to study the effect of starch on the cake resistance of the carbonatated liquor. These sugars were obtained from three geographical regions, labeled A, B and C. They were affined by our standard method and followed by the laboratory batch carbonatation. The cake resistances of the carbonatated liquors were determined for comparison. Results are summarised in Table 1.

Sample A3 was chosen to illustrate the effect of starch concentration on the cake resistance. Two more samples were made up with different portions of sample A3 and Tate & Lyle Sugars granulated sugar, which effectively diluted the quantity of starch and other impurities. Figure 3 shows the effect of starch content on the carbonatated liquor cake resistance. This figure shows that the cake resistance increases with starch content. The cake resistance of the carbonatated liquor made from this sample increases sharply when the starch content is greater than 200 ppm. It should be noted that this is the starch content of the affined sugar, not the whole raw.

Table 2 gives some typical starch contents of sugar determined before and after affination.

At Thames Refinery, the starch content of the melter liquor and the cake resistance of the corresponding carbonatated liquor was monitored in 1991-1992. These starch contents were below 200 ppm. Figure 4 shows the relationship between the starch content and the cake resistance. It appears that starch does not substantially reduce the cake resistance at concentrations of less than 150 ppm.

The effect of enzyme on cake resistance

Enzymes have been used to hydrolyse starch in sugar processing and refining. Termamyl™ is a liquid enzyme preparation containing heat-stable alpha-amylase produced by a selected strain of *Bacillus licheniformis*. The enzyme is an endoamylase which will hydrolyse 1,4-alpha-glucosidic linkages in amylose and amylopectin. Starch is therefore rapidly broken down to soluble dextrans and oligosaccharides.

Termamyl™ 120L was used in the laboratory to break down the starch prior to carbonatation. It was added at various concentrations to the 68 Brix affined sugar solution at 70°C with constant stirring. The resultant solution was carbonatated in the laboratory with a 0.5% lime dosage at 70°C. The cake resistances were measured and compared with the cake resistances obtained from enzyme treatment at various contact times and concentrations before carbonatation.

Figure 5 shows the effect of Termamyl™ 120L on the cake resistance of samples A2 and A3. The initial starch contents were 283 and 299 ppm for the affined sugars of A2 and A3 respectively. Experimental results indicate that the cake resistances dropped markedly within the first 5 minutes contact time at 10 ppm enzyme dosage. Only very small reduction in cake resistances was observed after 10 minutes contact time.

A much higher enzyme dosage of 100 ppm was also used on sample A3 and the cake resistance was found to be similar to the one obtained at 10 ppm with the same contact time of 15 minutes. A lower enzyme dosage of 5 ppm was used on sample A2 and the cake resistance was found similar to the one obtained at 10 ppm dosage with the same contact time of 10 minutes.

The effect of Termamyl™ 120L on the cake resistance of sugar sample B is shown in Figure 6. The affined sugars of B1 and B2 have starch contents of 308 and 258 ppm respectively. A 10 ppm treatment for 15 minutes gives a considerable reduction in cake resistances for both samples.

Figure 7 shows a similar effect of Termamyl™ 120L on the cake resistance for sugar sample C. A 10 ppm enzyme treatment for 15 minutes reduced the cake resistance from $151 \times 10^{10} \text{ cm}^{-2}$ to $92 \times 10^{10} \text{ cm}^{-2}$.

Table 3 shows the effect of Termamyl™ 120L on the cake resistance of the samples studied and illustrated in Figures 5, 6 and 7. When starch levels were high and interfering with the carbonatation process the cake resistance was reduced by at least a factor of two after enzyme treatment.

The relationship of filterability and cake resistance

It has been reported that the filterability of the affined sugar is related to the cake resistance of the carbonatated liquor when the same raw sugar was on the melt at Tate & Lyle Sugars Thames Refinery (3). However this study was carried out when all the raws received by Thames Refinery had less than 250 ppm (affined) starch content. It can also be shown that filterability is not linearly related to filtration rate and that the reciprocal of the square of the filterability is proportional to the cake resistance (4). These observations are valid only when suspended solids are the sole impedance to filtration, because the filterability test is conducted on the affined sugar solution without carbonatation.

Figure 8 shows the relationship between the filterability ($1/F^2$) of the affined sugar and the cake resistance of the corresponding laboratory carbonatated liquor. The correlation was obscured by the presence of starch in the affined sugar.

Figure 9 shows the same plot with the cake resistance measured after Termamyl™ 120L treatment. The starch effect on carbonatation was eliminated by enzyme and a linear relationship was observed. This is consistent with our work that the filterability test can be used to predict filtration performance of a carbonatation refinery when starch is not present in the raw above a threshold level of about 200 ppm.

DISCUSSIONS

The effect of amylose on carbonatation is well established. Amylose acts as a protective colloid coating the surface of the growing crystals. It may be visualised as wrapping itself around the growing crystallites. This produces a precipitate with poor agglomerate formation, and poor filtration characteristics (5).

The laboratory batch carbonatation developed can be used to study the effect of starch on the filtration characteristics of carbonatated liquor. The laboratory batch

carbonatation is performed under standard conditions and is suitable for comparison purposes.

The cake resistance data show that starch has an effect on the cake resistance. The magnitude of the effect of starch on cake resistance differs for different raw sugars. Samples collected from area A show that even in the sugar produced in the same geographical area the starch has a different effect on the cake resistance. This suggests that not only the concentration but also the type of starch, or more precisely the molecular weight of the starch, has different effects in carbonatation. In general the higher the starch (same type) content the worse the effect on the cake resistance of the carbonatated liquor. This is probably because of a higher percentage of the calcium carbonate surfaces are coated by the amylose molecules. It is also possible that the starch analysis method used cannot detect differences in the nature and form of the starch.

Experimental results indicate that Termamyl™ 120L is very effective in improving cake resistance even in high concentration of sugar solution at 68 Brix. These results indicate that an enzyme dosage of 5-10 ppm for 10-15 minutes will improve the cake resistance considerably. The removal of starch with Termamyl™ 120L has been studied by Tsang et al (7). Their experiments showed that the maximum hydrolysis obtained was 80% in 30 minutes at 70°C and pH 6.3 using 40 ppm Termamyl™ 120L at 60 Brix sugar solution. At higher enzyme dosage of 150 ppm, they also concluded that under similar conditions the percentage hydrolysis increases from 82-85 to 90-94 and 97-98 when the sugar solution concentration was decreased from 60 to 45 and 30 Brix.

Comparison of the present work with that of Tsang et al (10) suggests that a high degree of hydrolysis, say 80%, is not required to give an improvement in filtration, and hence a lower dosage of enzyme for a shorter time is quite sufficient to provide the improvement.

Figures 5, 6 and 7 and Table 3 show that the cake resistances after treatment with enzyme do not all come down to the same level. In fact the resistance after enzyme treatment for some affined sugar samples e.g. samples B1 and B2 are higher than the starting value for other affined sugars, i.e. before enzyme treatment. The reason for this is that affined sugars also have varying levels of suspended solids, and these also have an impact on cake resistance and filtration performance.

The Tate & Lyle Sugars filterability test gives a good indication of the filtration characteristics of an affined sugar. The test can measure filtration problems caused by suspended solids but cannot predict filtration problems caused by starch.

It has been derived and reported that the filterability ($1/F^2$) is linearly related to the cake resistance of the sugar solution (4). The filterability of the affined sugar ($1/F^2$) is also related to the cake resistance of the corresponding carbonatated liquor (3). However, Figure 8 does not show any correlation between the filterability of the affined sugar and the cake resistance of the corresponding carbonatated liquor for the raw sugars tested in this work. The cake resistance measured represents the total impedance contributed by suspended solids and starch interference.

Once the starch was hydrolysed by Termamyl™ 120L the impedance resulting from the starch effect was eliminated, and suspended solids became the sole filtration impedance. Figure 9 shows a linear relationship between the filterability ($1/F^2$) of the affined sugar and the corresponding cake resistance of the carbonatated liquor. Linear regression gives a correlation coefficient R^2 value of 0.99. With the effect of starch eliminated the filterability test, which gives the value of filterability F , now relates well to cake resistance, and hence to the rate of filtration. However, this also shows that if a raw is difficult to filter because of a high level of suspended solids and a high level of starch, it will be improved by enzyme treatment, but will still be difficult to filter.

A knowledge of both the suspended solids level measured by the filterability test, and the amount of starch, measured by analysis, are necessary to predict the filtration characteristics of raw sugar. The filterability test can be used to predict carbonatation refinery filtration performance when processing raw with low starch content, <200 ppm affined sugar starch.

CONCLUSIONS

Starch reduces sugar filterability by interfering with calcium carbonate growth. This paper describes and uses a laboratory carbonatation method to quantify the effects on a number of samples of raw sugar. The threshold level of starch in the affined sugar, measured by the technique described by Plews (11) below which starch has a relatively small effect on carbonatation is about 200 ppm.

Alpha-amylase enzyme (Termamyl™ 120L) was found effective to improve the filtration rate of the carbonatated liquor when starch causes a problem. A 5-10 ppm of the enzyme can be applied to a 68 Brix sugar solution at 70°C for 10-15 minutes. The cake resistance of the carbonatated liquor drops considerably after the enzyme treatment showing that even at a high Brix the enzyme is still very effective. It is likely that a complete hydrolysis of the starch is not necessary to obtain a very considerable improvement in filterability.

A function of the filterability of the sugar ($1/F^2$) has a linear relationship with the cake resistance of the corresponding carbonatated liquor. However, when starch is present it will obscure this correlation. Treatment with alpha-amylase will remove the effect of the starch and the linear relationships, caused by the suspended solids present, still holds.

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APPENDIX 1

Standard Method Procedure

1. Prepare sample solution and adjust to $65 \pm 0.05\text{Bx}$ in a plastic bottle. Add $0.5\% \pm 0.002\%$ (on solid) Celite 505, stopper and heat in 72°C water bath for 45 minutes with occasional shaking.
2. Assemble pressure filtration unit, mount in jacket and attach an outlet valve to the outlet of the filter unit in a closed position.
3. Circulate water through jacket and maintain at 70°C , check for leakage of filtration unit.
4. Transfer sample to the filter through a funnel, start stop watch simultaneously and allow to stand for 3 minutes before filtration.
5. Connect filter to the pressure supply cylinder when 2.75 minutes has elapsed.
6. At 3 minutes, raise the applied pressure instantly (pre set at 60 psi) and simultaneously open the stop valve.
7. Collect filtrate in a measuring cylinder. Record volume and time.
8. Finally, mix filtrate, cool and measure Brix to check leakage.
9. The volume of the filtrate collected at 5 minutes is used to calculate filterability.

$$\% \text{ Filterability} = \frac{\text{Vol. of Filtrate of Sample}}{\text{Vol. of Filtrate of Filtered Gran. Sugar Soln.}} \times 100$$

APPENDIX 1 (continued)

Standardization of Filterability

1. Prepare a granulated sugar solution and adjust to 65 ± 0.05 Brix.
2. Add $0.5\% \pm 0.002\%$ (on solid) Celite 505, heat in 72°C water bath for 45 minutes and filter at 70°C with Whatman GFA paper.
3. The filtrate is then used for standardization. Proceed as described in Standard Method.
4. The filterability of a filtered granulated sugar solution is taken at 100%.

Table 1. Effect of starch content on cake resistance.

Sample	Starch ppm	Cake resistance cm^{-2}
A1	83	48×10^{10}
A2	283	55×10^{10}
A3	299	122×10^{10}
A4	331	74×10^{10}
B1	308	496×10^{10}
B2	258	297×10^{10}
C1	166	72×10^{10}
C2	209	151×10^{10}

Table 2. Typical starch contents of raw and affined sugars.

Sample	Starch ppm raw sugar	Starch ppm affined sugar
1	357	302
2	267	205
3	244	222
4	226	188
5	136	75

Table 3. Effect of Termamyl™ 120L. (Laboratory batch carbonatation)

Sample	Filterability %	$r \times 10^{-10} \text{ cm}^{-2}$	$r^* \times 10^{-10} \text{ cm}^{-2}$
A1	43	48	46
A2	77	55	23
A3	67	122	26
A4	80	74	17
B1	26	496	182
B2	27	297	153
C1	42	72	67
C2	36	151	92
TLS Gran	98	12	---

* After Termamyl™ 120L treatment

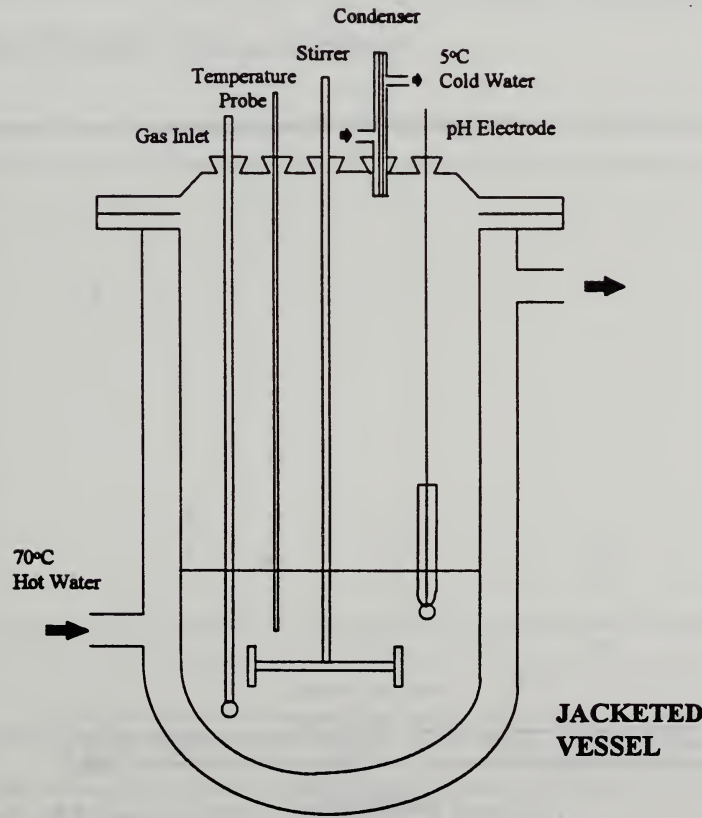


Figure 1. Batch carbonatation apparatus.

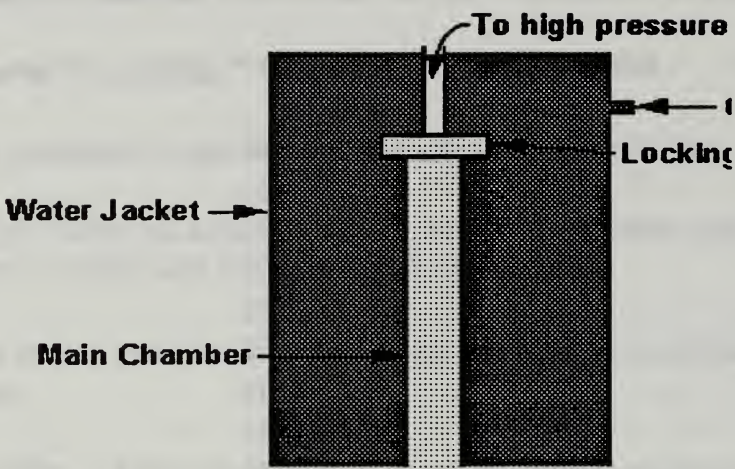


Figure 2. Diagram of filtration test equipment.

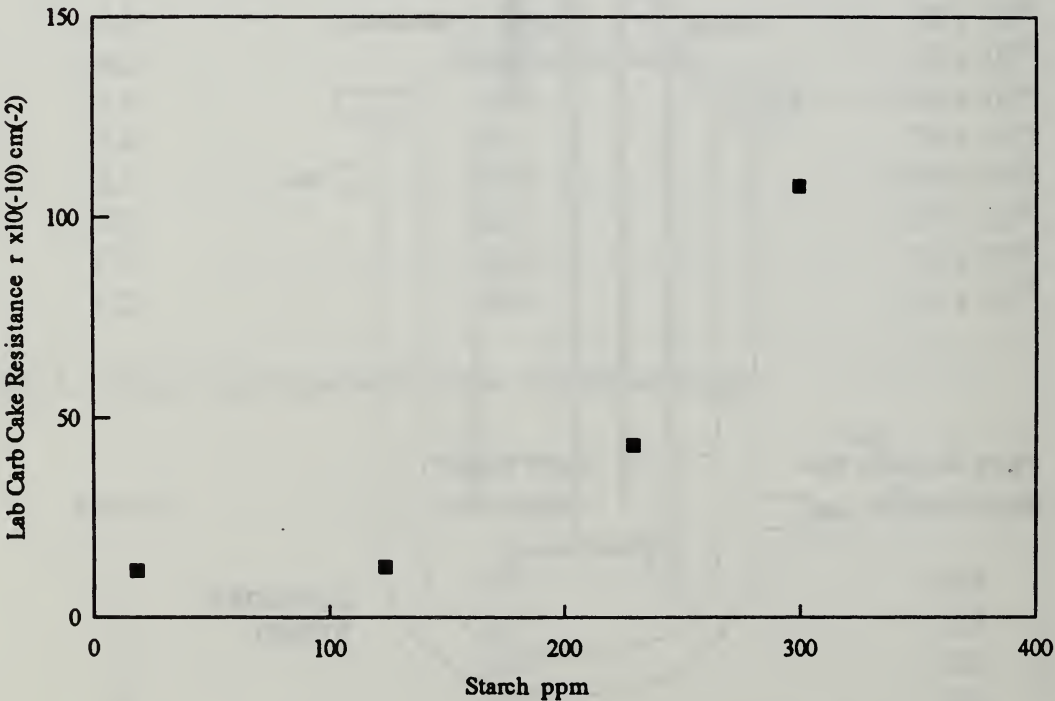


Figure 3. Effect of starch (Sample A3).

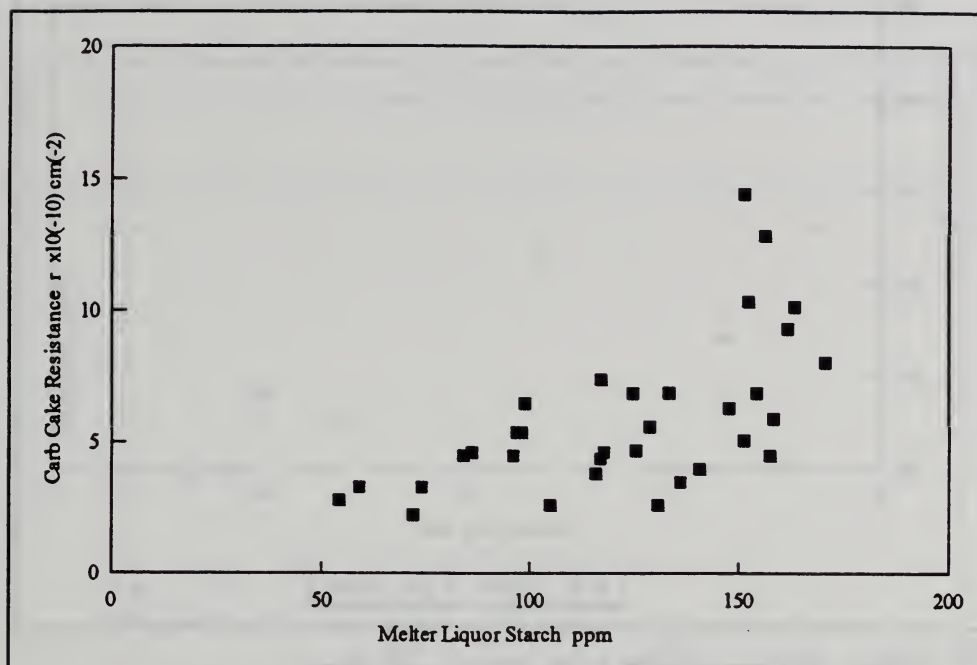


Figure 4. Effect of starch (Thames Refinery).

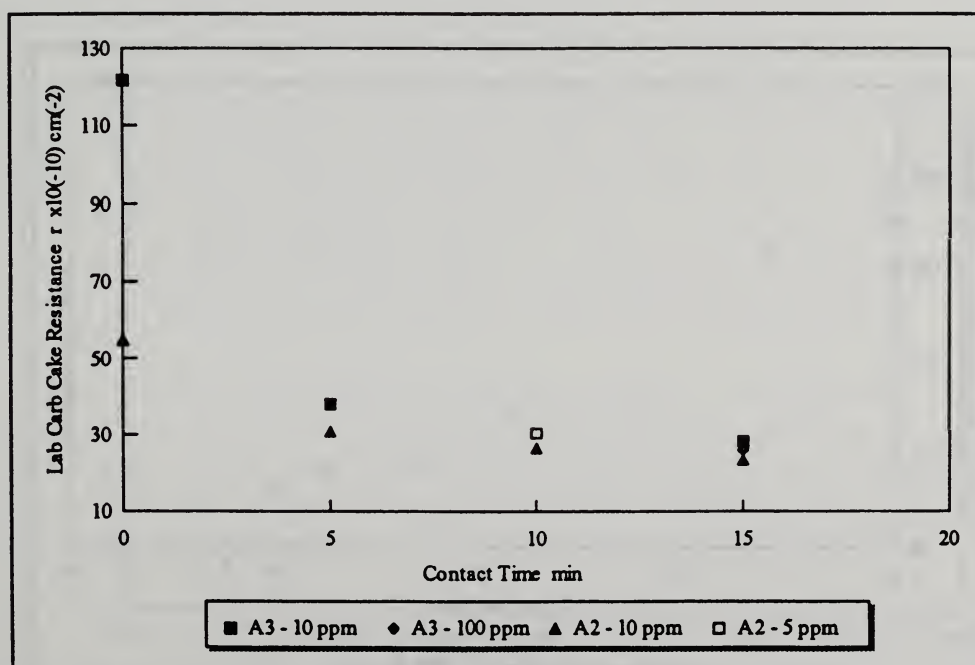


Figure 5. Effect of Termamyl™ 120L (70°C - 68 Brix).

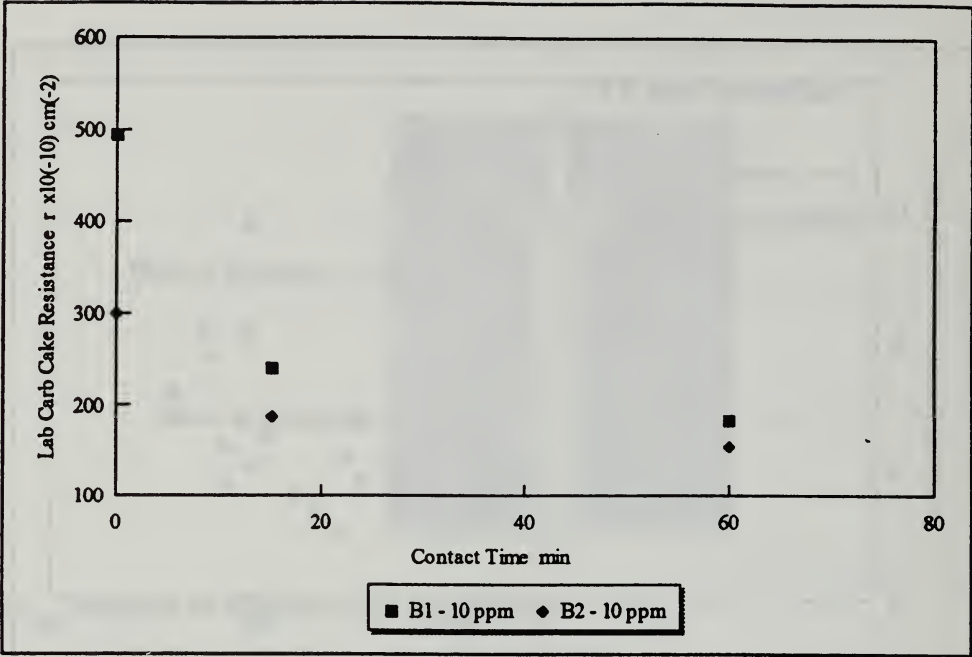


Figure 6. Effect of Termamyl™ 120L (70°C - 68 Brix).

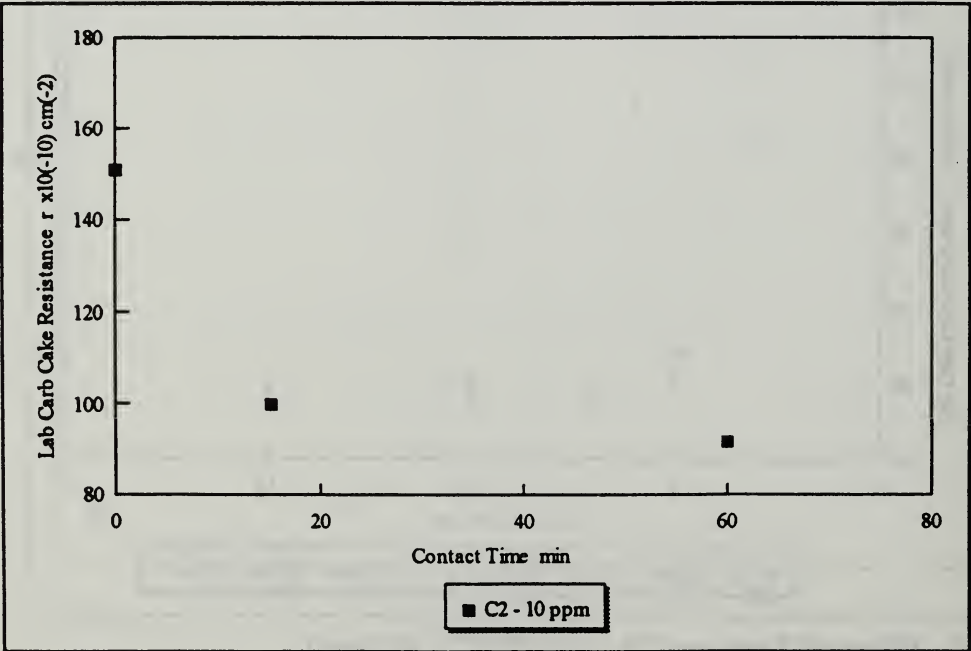


Figure 7. Effect of Termamyl™ 120L (70°C - 68 Brix).

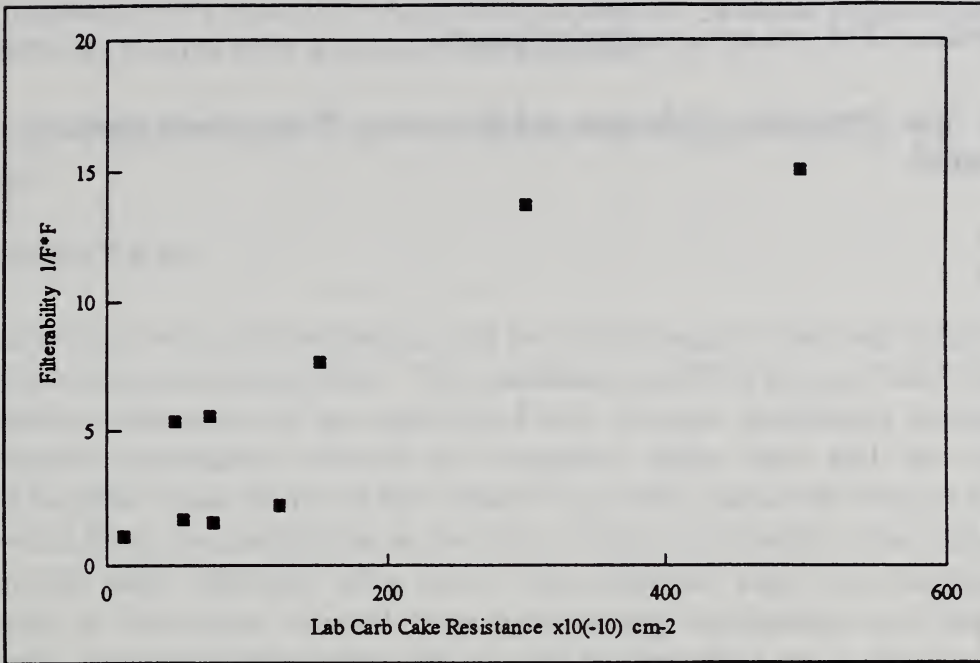


Figure 8. Filterability and cake resistance.

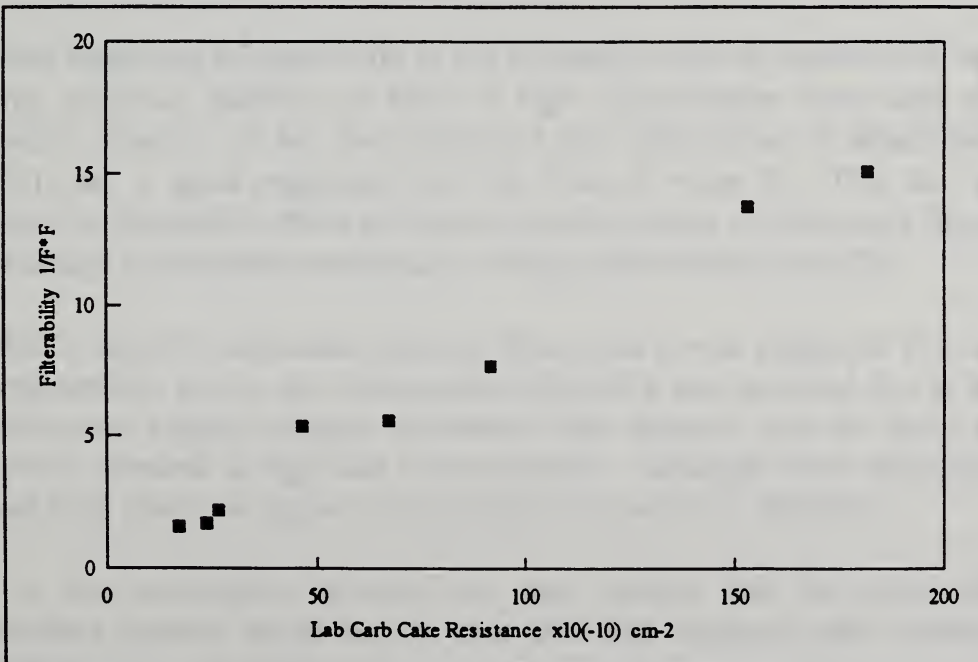


Figure 9. Filterability and cake resistance (TermamylTM 120L treated).

DISCUSSION

Question: Did you try any other enzymes?

Lee: Yes. Proteinase, Pulluzyme and Nervanase. Nervanase is similar to Termamyl.

INFLUENCE OF CALCIUM AND SUCROSE IN SUGAR COLOURANTS REMOVAL FROM ION EXCHANGE RESINS

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INTRODUCTION

It is known that two main mechanisms are involved in sugar colourants fixation into strong base anion exchange resins. One mechanism involves an ionic bond between the anionic colourant and the resin fixed ion. Another mechanism involves an hydrophobic interaction between the colourant apolar part and the styrenic divinyl-benzenic resin matrix. It was observed (2) that colourants fixation to resin can switch from one mechanism to the other during regeneration. These facts can explain the high efficiency of a resin, when new, as sugar decolourizer, the difficulty of colourants removal from resin during regeneration and the rapid efficiency decrease during resin life. It will be important to understand these mechanisms and avoid the ineffective colourants removal from resin in order to use the maximum efficiency of ion exchange resins.

Since the beginning of application of ion exchange resins as decolourizer in sugar industry, aqueous solutions of NaCl at high concentration have been used as regenerant solutions. It has been observed that NaCl alone or alkalinized with NaOH is not a good regenerant for this kind of resins (6). This fact can be explained by the switch effect and due to the decreasing of colourants desorption fixed through hydrophobic mechanisms at high salt concentrations (2).

In order to improve colourants removal from resin it was suggested (1) to make resin regeneration at two salt concentrations. In fact it was observed that at low salt concentrations slightly charged colourants were released, and the more anionic ones were released at high salt concentrations. Although more colourants are released from resin this regeneration process is not yet very effective.

Due to the mechanisms involved we can preview that the most effective regeneration system would be the one that will suppress both mechanisms, simultaneously.

Therefore we can use a high salt concentration and improve the removal of hydrophobic colourants using an organic solvent, as ethanol, mixed with salt. With this mixture, salt will remove the ionic colourants and ethanol will decrease the hydrophobic inter-action effect. This was tried using a mixture of NaCl at 100g/l and Ethanol at 20% v/v (2) with a great increase of colourants removal from resin. Although efficient this process is not economical if applied to each regeneration.

Other possibility is to use low salt concentrations and improve resin ion exchange mechanism to remove the high charged anionic colourants. At low salt concentration, apolar colourants are easily removed, but low chloride ion concentration is not enough to remove high charged anionic colourants efficiently. It will be then necessary to dislocate the equilibrium of the ion exchange mechanism to the regeneration side, that is the exchange of chloride ion by the colourant anion fixed to the resin. It will be proved in this paper that adding calcium ions to the alkaline salt solution resin regeneration improves greatly. A possible formation of a colourant-calcium complex can cause the dislocation of the regeneration equilibrium and explain this fact.

In this paper we will study the influence of calcium salts in the sugar colourants removal from ion exchange resins. In order to maintain calcium in solution at a high alkalinity, sucrose was added to the solution. Calcium saccharate will be formed maintaining calcium salts in solution.

MATERIAL AND METHODS

For the tests described herewith we used a strong base divinyl benzenic resin type in the chloride form (Amberlite IRA900C).

Batch tests

Resin for these tests was previously charged with colourants from carbonated liquor of Oporto Refinery. This was achieved by contacting the resin in a batch way 4 x 1 liter of liquor per liter of resin during 4 hours at 40°C, with agitation. After this procedure, resin was washed with distilled water and air dried. This resin will be referred to as charged resin.

For batch tests, 2.5g of charged resin was weighted to a 100ml closed flask and mixed with 50ml of test solutions during 4 hours, with agitation, in a water bath at 40°C. After this time resin was removed from solution through vacuum filtration.

Tests of colourants precipitation with calcium

100ml of resin effluent solution was contacted with agitation at 60°C with 50ml of a slurry containing $\text{Ca}(\text{OH})_2$ at a concentration equivalent to 10g/l of CaO. After 30 minutes solution was filtered through Whatman 42 filter paper and a solution attenuation at 420nm was measured.

Tests in column

Resin (100ml) was placed in a water jacket Pharmacia column. Resin was charged with 40BV of colourants solutions at a flow rate of 3BV/hour at 40°C. Column was heated with hot water at 40°C during charge and regeneration. Resin regeneration was made with 3BV of regenerant solution at a flow rate of 2BV/hour at 40°C. Colourants solutions comprise carbonated liquor from Oporto Refinery, diluted with distilled water (3:1), and aqueous solutions of colourants Caramel, Melanoidin and Hexoses Alkaline Degradation Products (HADP). Synthetic colourants were prepared as described in the literature (4) and were diluted with distilled water until an attenuation of 600 ± 50 at 420nm, and pH adjusted to 8.5.

Analysis

Absorbancies were measured at 420nm, in a cell of 1cm. Solutions were adjusted to pH 9.0 and filtered through 1.2 μm filters before readings. Attenuances were calculated as equivalent to $(\text{Absorbance} \times 1000) / \text{cell length}$. For analysis of absorbancy of regenerant effluents, a solution of EDTA at 100g/l was mixed (1:1) with samples in order to avoid calcium precipitation.

RESULTS AND DISCUSSION

Colourants desorption in presence of NaCl

Charged resin was put in contact, as described, with sodium chloride solutions from 0 to 2.0N. Results of solutions attenuances are indicated in Table 1 and presented in Figure 1. As it is observed, desorption of colourants from resin increases with

NaCl concentration. The curve slope is higher at values lower than 1.0N. After this concentration the increase of colourants removal with the increase of chloride concentration is not so high.

Figure 1 also shows the attenuances of solutions when NaCl containing 0.5% of NaOH was contacted with charged resin. An increase of colourants desorption was observed, as was expected, being higher at higher salt concentrations. This can be explained by the fact that at high alkalinity colourants tend to be anionic and will be removed by ion-exchange with chloride ion in solution. Some of these colourants, at a lower pH will be maintained neutral and will be fixed to the resin matrix by hydrophobic inter-action.

The influence of sucrose in colourants desorption from resin was tested adding sucrose at 20°Brix and NaOH at 0.5% to NaCl solutions. Results are presented in Table 1 and Figure 1. As it is observed the influence of sucrose on colourants desorption is small.

In order to test the influence of calcium on colourants removal we need to have calcium in solution at high alkalinity. This was achieved using calcium hydroxide in a 20°Brix sucrose solution. It is known that solubility of lime increases with sucrose concentration due to formation of mono- and di-calcium saccharate (5) (Figure 3). Calcium solubility decreases with temperature as saccharate hydrolysis increase with it.

When alkanisation was caused by $\text{Ca}(\text{OH})_2$ instead of NaOH, a great increase of colourants removal was observed (Table 1 and Figure 1). Tests were made using different concentrations of $\text{Ca}(\text{OH})_2$, equivalent to 5g/l, 10g/l and 20g/l of CaO. It was observed that colourants removal increases with $\text{Ca}(\text{OH})_2$ in solution.

Colourants desorption in presence of CaCl_2

In this serial of tests, colourants from charged resin were removed using CaCl_2 instead of NaCl. Results are presented in Table 2 and Figure 2. It was observed that CaCl_2 alone remove more colourants than when NaCl is used.

The presence of sucrose has a small effect when mixed with CaCl_2 (Figure 2). However, when NaOH was added to alkalize the solutions, a great increase of colourants removal was observed. In Figure 4 is indicated the relation of

attenuances when CaCl_2 is used related to the ones when NaCl was used. As observed, a higher difference exist at low salt concentrations indicating that the influence of calcium ion is more evident at these concentrations.

As in the previous tests, the presence of Ca(OH)_2 in a 20°Brix sucrose solution increases colourants desorption. However, when CaO concentration was increased to 20g/l, a decrease of colourants desorption was observed. In order to evaluate the optimum CaO concentration to obtain a maximum colourants removal, tests with increased concentrations of CaO were performed. In these tests CaCl_2 was maintained at 1.5N and sucrose at 20°Brix. Results are presented in Figure 5. A maximum of solution attenuance is observed at a CaO concentration of 12g/l. This concentration corresponds to a relation of molecules of sucrose to Ca(OH)_2 of exactly 3:1.

Colourants precipitation in presence of calcium: influence of sucrose

It is known that anionic colourants precipitate in presence of calcium hydroxide (1). However, if sucrose is present in the solution containing the colourants the precipitation does not occur. But, when sucrose is added to the solution after the precipitation, the precipitate is not be greatly affected. This was tested using an effluent containing anionic colourants removed from the resin (HDE). HDE was contacted with Ca(OH)_2 , as described earlier, with the formation of a calcium/colourant precipitate. Attenuances at 420nm and decolourization observed are presented in Table 3. Sucrose was added to the solution with the precipitate in a quantity equivalent to 20°Brix solution. The solution was heated with agitation as before. It was observed that the precipitate practically was not affected by the presence of sucrose.

The same test was performed with a mixture of HDE and sucrose obtaining a solution at 20°Brix. It was observed that with this solution the precipitation did not occurred.

The fact that the presence of sucrose suppress the calcium/colourant precipitation is important because the occurrence of such precipitation could be detrimental to the resin. Possibly a complex calcium/colourant is formed that is maintained in solution by the presence of sucrose.

Tests with resin in column

The previous results indicate that a mixture of sodium or calcium chloride and calcium hydroxide in a sucrose solution can with advantage substitute the normal regeneration using a alkaline solution of sodium chloride.

As observed in Figure 6 even with a low chloride ion concentration (equivalent to 10g/l of NaCl) we have the same desorption power as when a high concentration of NaCl (100g/l) alkalized with 0.5% NaOH is used.

This new regeneration system (patent pending, Bento, 1995) was used to regenerate a strong base anionic resin (Amberlite IRA 900C) in 100ml column, as described earlier.

Resin was charged with solutions containing the following colourant solutions: Caramel, Melanoidin, HADP and carbonated liquor.

Two processes of resin regeneration were used:

Salt regeneration: 3 BV of NaCl at 100g/l + NaOH at 0.5%

Saccharate regeneration (SC): 3 BV of mixture(*) + CaCl₂ 0.6N

(*) - Sucrose at 150g/l and Ca(OH)₂ at 10g/l of CaO.

Results of colourants adsorbed to the resin and removed during regeneration (considering absorption at 420nm at pH 9.0 proportional to quantity of colourant) are indicated in Table 4.

Caramel, the colourant that was least retained on ion exchange resins, in this test, was also, with HADP, difficult to remove with alkaline NaCl solutions during regeneration. With SC regeneration more 9% of caramel colour was removed (Figure 7). Melanoidin was one type of sugar colourant that was well removed by ion exchange resin and better regenerated with NaCl than Caramel and HADP colourants. With SC regeneration practically all melanoidin colourant fixed to resin was removed. Using this process 27% more of melanoidin colour was removed with this regeneration process (Figure 8).

HADP colourant was well retained to resin but was not well removed with alkaline NaCl solutions. When SC regeneration was used a much more colourant removal from resin was obtained. Using this process 53% more of HADP colour was removed when SC regeneration was used (Figure 9).

With carbonated liquor we used an excess regenerant, 6 BV, and the results are presented in Figure 10. It is observed that more colourants were released with SC regeneration than with alkaline NaCl solution. Exactly 38% more of colour was removed during regeneration. Even when a lower concentration of CaCl_2 (0.3N) was used a better colourant removal was obtained (60.9%).

With these results a serial of tests were performed in a column containing one liter of resin. After 46 cycles resin decolourization was maintained over 90%. Sucrose and salts were partially recovered using tangential filtration techniques (3).

CONCLUSION

Sugar colourants are fixed to ion exchange resins through two mechanisms that can act to the same colourant molecule. In order to improve regeneration efficiency it will be necessary to suppress both mechanisms simultaneously. This was achieved adding calcium ions to the alkaline regenerant solution. Calcium will form, possibly, a complex with sugar colourants and will dislocate the ion exchange equation to the regeneration side. To maintain this complex and calcium hydroxide in solution it is necessary to add sucrose to the regenerant solution. With a mixture of chloride ions, calcium ions and sucrose in an alkaline medium a much better colourants removal from resin was achieved. Tests with synthetic colourants and with carbonated liquor indicate that this regeneration process can be used successfully to decolourize resins used in factory and sugar refining processes. With this process even with a low salt concentration resin can be regenerated efficiently. Thus, chemicals consumption and pollution can be reduced greatly. The success of this new system will depend on sucrose recovery from coloured effluents.

ACKNOWLEDGMENTS

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Table 1. Attenuation of solutions - mixtures of NaCl.

NaCl						
		Sucrose 20° Brix				
NaCl		NaOH	NaOH	Ca(OH) ₂	Ca(OH) ₂	Ca(OH) ₂
		0.5%	0.5%	5g/l CaO	10g/l CaO	20g/l Cao
0	20	11	14	60	114	204
0.2N	140	103	164	828	1140	1252
0.4N	284	338	452	1340	1552	1574
0.6N	414	656	692	1564	1724	1708
0.8N	510	870	890	1686	1731	1782
1.0N	594	1038	1072	1700	1743	1896
1.5N	648	1214	1292	1684	1746	2028
2.0N	754	1212	1340	1650	1815	1980

Table 2. Attenuation of solutions - mixtures of CaCl₂.

CaCl ₂						
		Sucrose 20° Brix				
CaCl ₂			NaOH	Ca(OH) ₂	Ca(OH) ₂	Ca(OH) ₂
			0.5%	5g/l CaO	10g/l CaO	20g/l Cao
0	20	20	14	14	114	204
0.2N	262	340	720	1210	1424	1328
0.4N	502	640	1320	1658	1758	1690
0.6N	682	776	1498	1854	2040	1812
0.8N	864	876	1512	1938	2016	1824
1.0N	902	956	1590	1962	2058	1884
1.5N	908	1026	1620	1914	2070	1851
2.0N	760	904	1630	1881	2010	1758

Table 3. Influence of sucrose in anionic colourants precipitation with $\text{Ca}(\text{OH})_2$.

	Attenuation	% Dec.
HDE *	3630	-
HDE + $\text{Ca}(\text{OH})_2$	1138	68.6%
(HDE + $\text{Ca}(\text{OH})_2$) + Sucrose	1374	62.1%
(HDE + Sucrose) + $\text{Ca}(\text{OH})_2$	3430	5.5%

* Distilled water (50:100) was added to have the same dilution.

Table 4. Comparison of decolourization and colourants removal from resin using two regeneration processes.

	% dec. *	NaCl + NaOH	SC
Caramel	69.4%	55.0%	60.2%
Melanoidin	96.7%	75.7%	96.5%
HADP	97.6%	54.3%	83.3%
Carbonated liquor	93.6%	54.2%	75.1%

* average

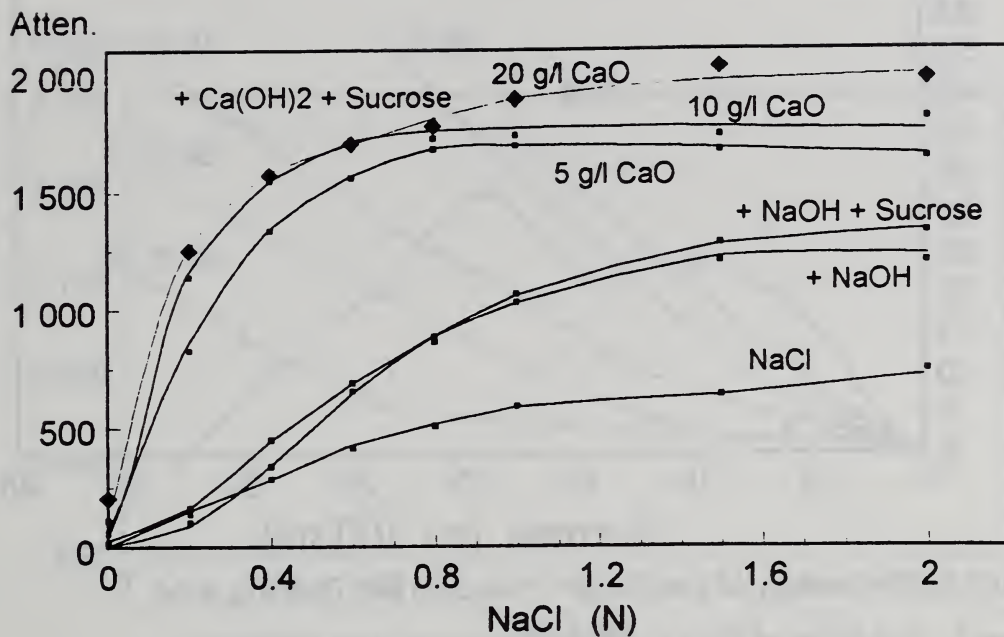


Figure 1. Colourants removal with mixtures of NaCl.

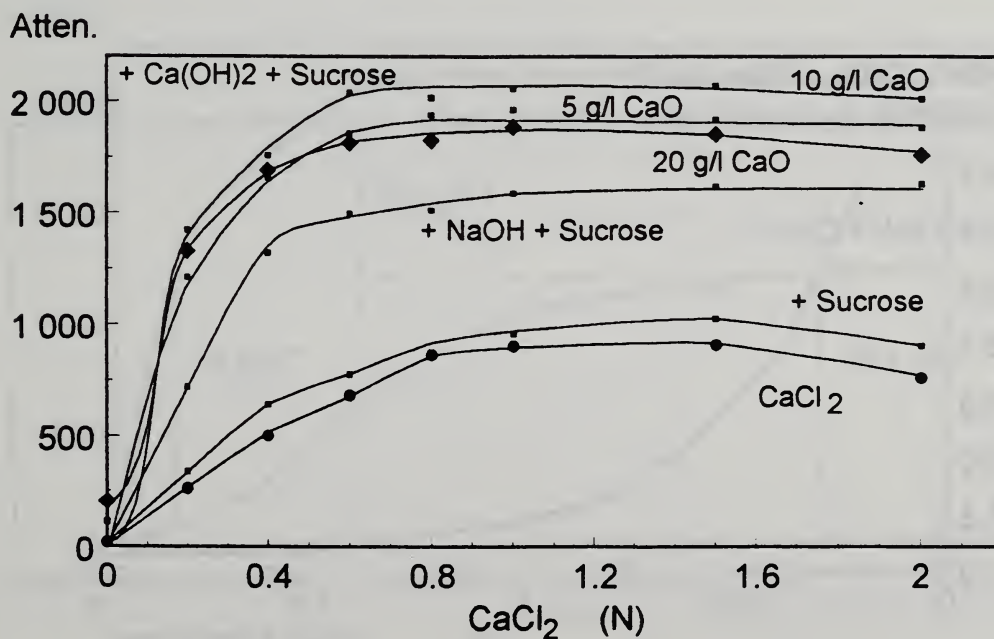
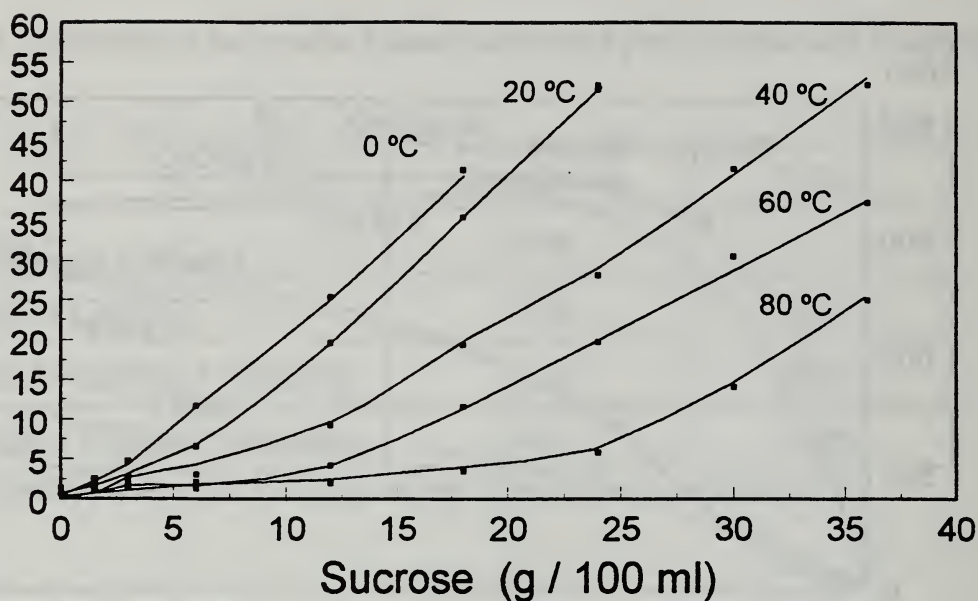


Figure 2. Colourants removal with mixtures of CaCl₂.



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Figure 3. Solubility of CaO at different sucrose concentrations.

Atten. CaCl_2 / Atten. NaCl

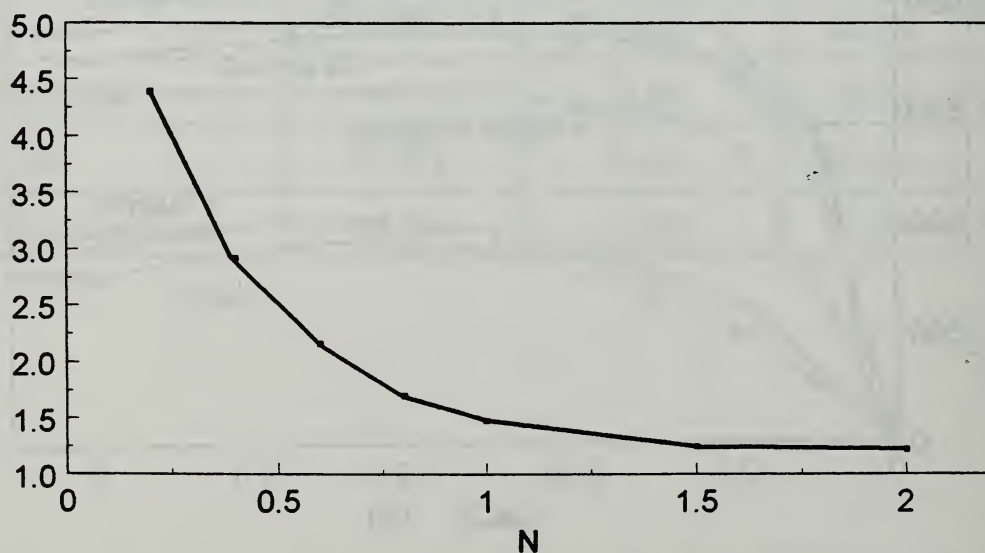


Figure 4. Regeneration with $\text{CaCl}_2/\text{NaCl} + \text{NaOH} + \text{sucrose}$.

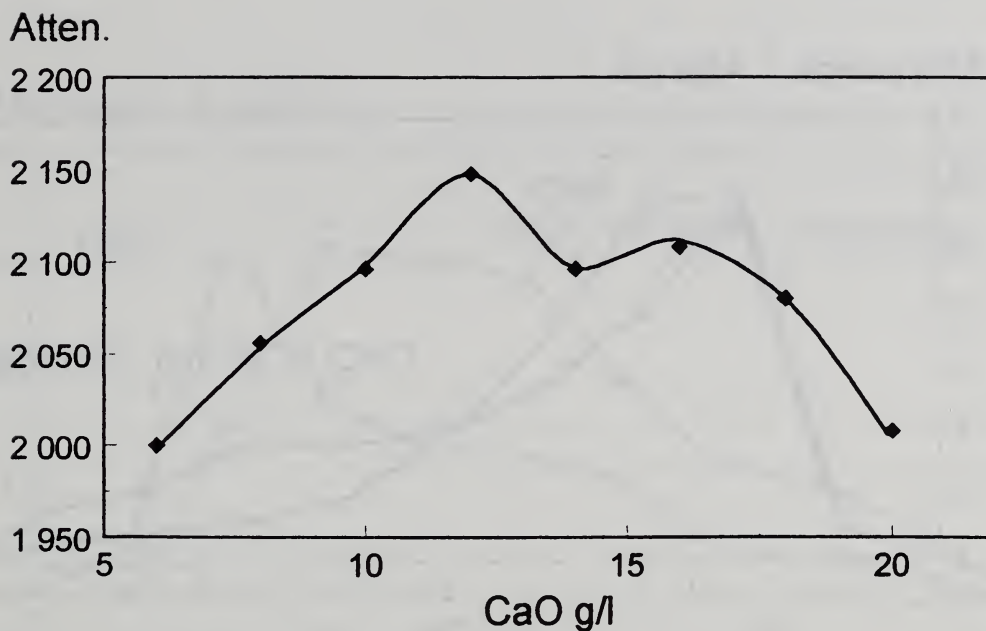


Figure 5. CaCl_2 1.5 N + sucrose 20° Brix.

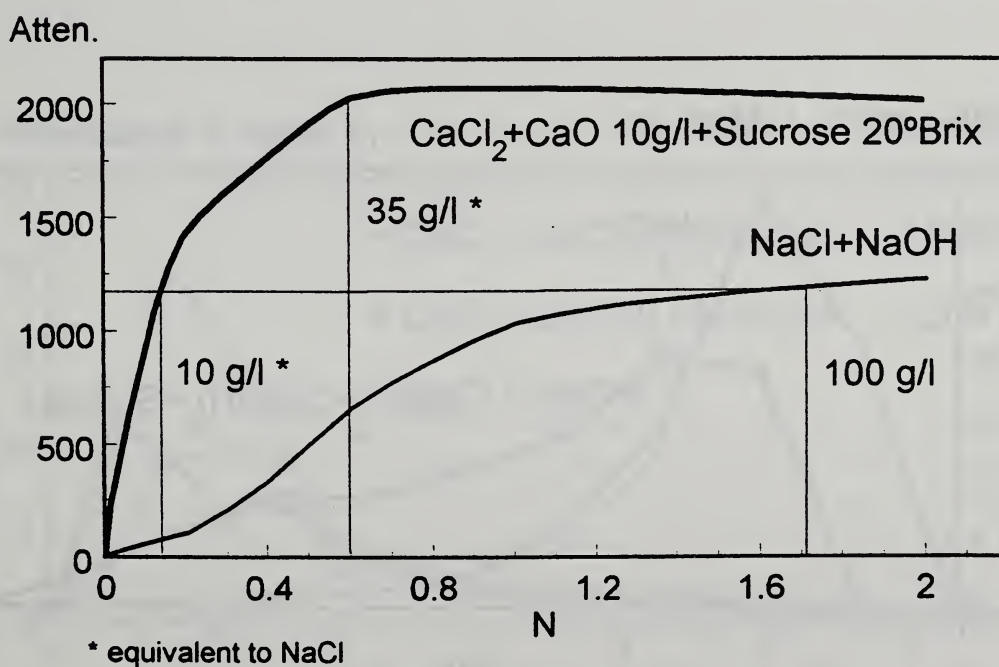


Figure 6. Comparison of two methods of colourants removal from resins.

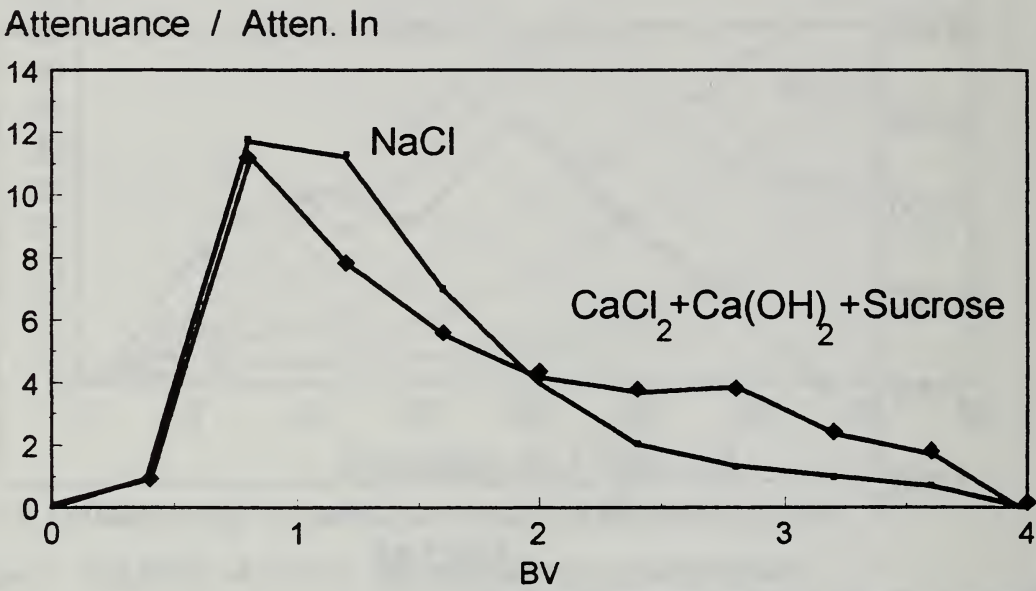


Figure 7. Caramel.

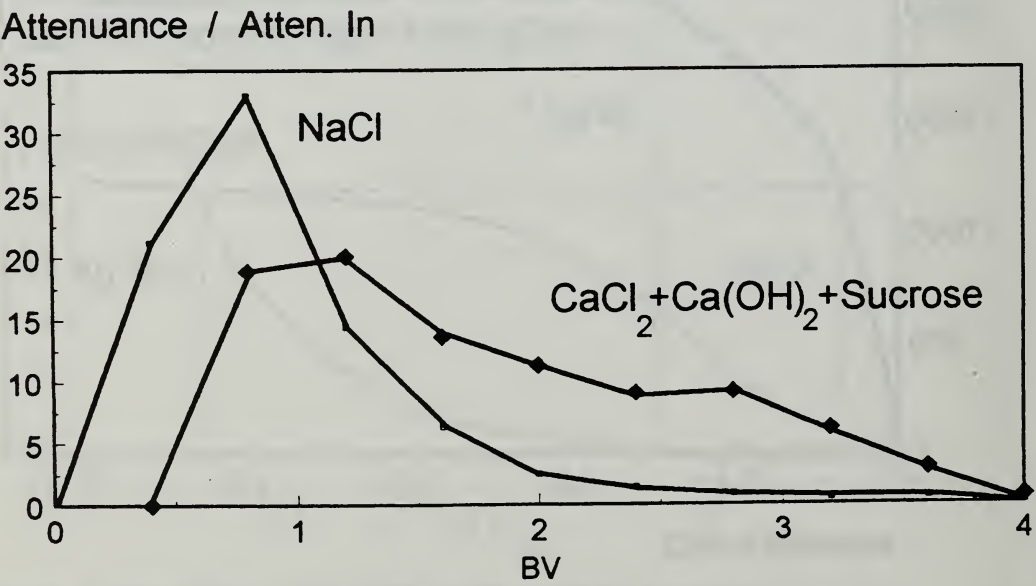


Figure 8. Melanoidin.

Attenuance / Atten. In

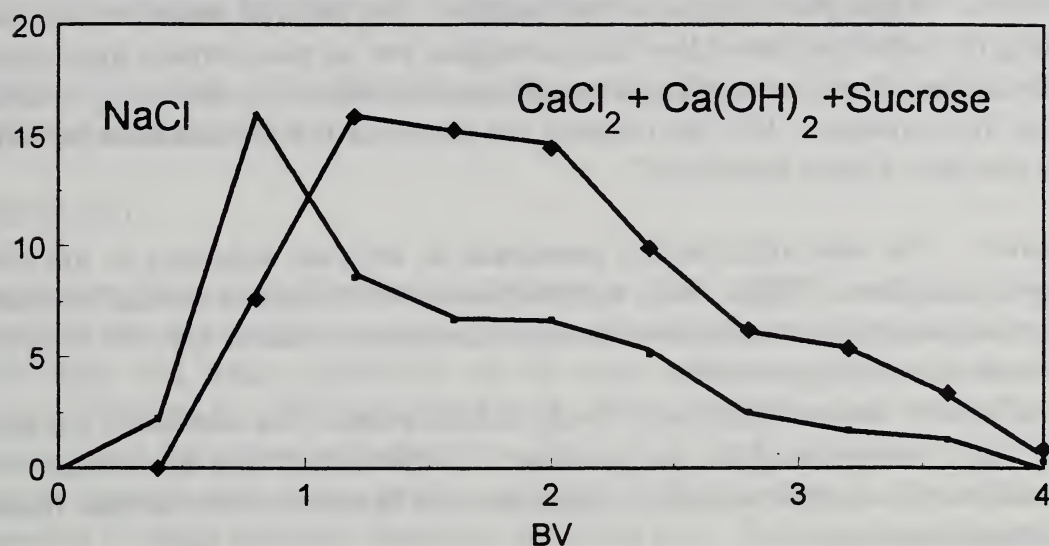


Figure 9. HADP.

Attenuance / Atten. In

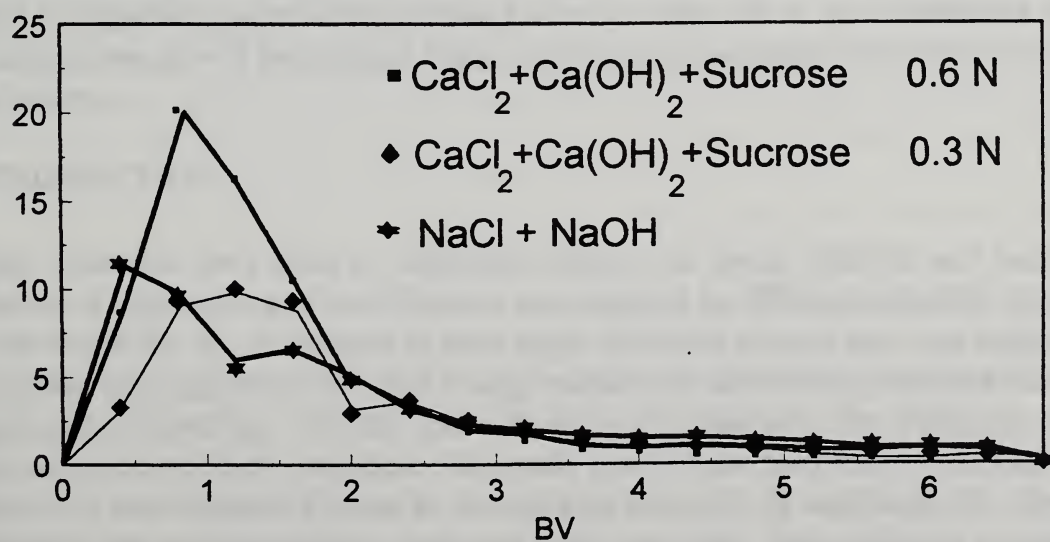


Figure 10. Carbonated liquor.

DISCUSSION

Question: About your last slide, where you are proposing ultrafiltration to regenerate ion-exchange resin regenerant, to send colorant to molasses: we have noticed, on gel permeation chromatography, that colored materials are carried along by carbohydrates at low ionic strengths, but as you increase ionic strength, some colorant can be separated from polysaccharides. You are trying to separate salts from colorant. Will the colorant not permeate the ultrafiltration membrane? Do you have further comments?

Bento: We used ultrafiltration membrane to separate colorants in the effluent regenerant (Bento 1996). With a membrane of 4000 Daltons we had a separation of colorants to the retentate with eight times more colour (at 420 nm) in the retentate than in the permeate.

Chloride ions are distributed evenly in both sides of the membrane but calcium are more concentrated in the retentate. I believe that this fact is due to the formation of a complex calcium-colourants and therefore more calcium remain in the retentate.

SEPARATION AND IDENTIFICATION OF CONSTITUENTS OF COLOMBIAN RAW SUGAR

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ABSTRACT

The major classes of colorants in Colombian raw sugar were isolated using methanol as an extracting agent. The crude extract was partitioned between chloroform and water; extraction of the main colorant components from the aqueous fraction by ethyl acetate followed. This last fraction was rich in several phenolic acid derivatives, carbohydrate-related compounds and glyceric acid. The analysis for each extract was performed by GC-MS according to a procedure for phenolics in sugar products described by SPRI (5). Fractionation of an acidic aqueous solution of raw sugar by XAD-2 Amberlite resin showed, as major components, phenolic compounds with slightly and moderate acidic groups eluted by sodium carbonate solution, and methanol, respectively.

Further studies like the effect of cane burning and the impact of tops and trash on color in sugarcane juice were estimated very recently and it was established that burning alone as well as tops and trash contributed to increased cane juice color in the factory.

INTRODUCTION

Sugar colorants have been an important subject for many chemists and various schemes of separation and identification were studied by different scientists around of the world (2, 6). Colorants in cane sugar products include not only naturally occurring plant pigments but also a large number of substances produced during sugarcane processing. Of the general types of colorants, the phenolics and flavonoids come from the plant. In recent years these have been described by Clarke (2) and Godshall (5) and in Australia by Paton (6, 7) and Smith (9). Some phenolics are colorless when extracted from the plant, but oxidation enzyme-catalized reactions produce colorants by polymerization of the generated quinones

or further reactions with amino acids or amino compounds (1, 8). The most important colorants of raw sugar developed in factory processes are melanoidins, caramels and alkaline degradation products of fructose (2, 8).

In addition to these classes of colorants, there are several colorless compounds known as color precursors, which can form color in the factory processes. These include amino acids, many hydroxyacids and aldehydes as well as iron, which complexes to make colored compounds (1, 6). Simple, practical analytical procedures for separation and identification have been developed (1, 6, 8). Godshall and Roberts (5) reported that the non-ionic macroporous polystyrene resin XAD-2 can be used to obtain selectively a fraction from sugar with a high concentration of colors and flavors. They also found that the combination of XAD-2 extraction of sugars, followed by chloroform extraction and GLC analysis, provided a sensitive method to evaluate the minor constituents, of sugar. Other techniques such as paper chromatography and paper electrophoresis have been applied in the separation of many color-contributing substances present in raw sugar (1, 6).

The effects of cane tops and field trash to the cane quality have been reported by many investigators (3). Results obtained in South Africa by Lionnet indicated that trash as leaves and tops contributed significantly to color on mixed juice (3) and this factor in turn influences the extent of color formation during processing of raw sugar.

Color is a major determinant factor of the quality of raw sugar and further knowledge on color components is necessary as a first step in improving the quality of raw sugar from Colombia.

MATERIALS AND METHODS

Separation of colorants from raw sugar

Raw sugar (600 g) from Manuelita Mill, located in the Cauca Valley of Colombia, was ground up and mixed well with 650 ml of methanol; the mixture was covered and kept at room temperature for 90 h. The methanol solution was separated by filtration from the suspended particles, and the separated solution was concentrated under reduced pressure. The remaining syrup was evaporated and then partitioned between chloroform and water. The aqueous fraction was extracted with ethyl

acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated to a syrup (fraction A). The chloroform extract was reduced to a small volume (rotary evaporator) and partitioned between light petroleum and methanol-water (9:1). Concentration of these extracts gave after removal of solvent under reduced pressure and drying with anhydrous sodium sulphate a methanolic extract (fraction B) and petroleum ether extract (fraction C) (Figure 1).

The ethyl acetate extract (A), methanolic extract (B) and petroleum ether extract (C) were evaporated to dryness in 2-ml vials and dissolved in a minimum amount of pyridine.

The GC-MS analysis of the trimethylsilyl derivatives (TMS) for each extract was performed according to a procedure for phenolics in sugar products described by SPRI (5).

Effects of Cane Burning to Color on Sugarcane Juice

Commercial trials with the MZC 74275 variety under conditions of burning and not burning (green harvested cane) was performed during four different weeks. Clean burnt and unburnt cane was delivered, immediately after harvesting, to the Mayagüez Sugar Mill located in the Cauca Valley. Fifteen samples of first expressed juice from each treatment were collected and analyzed in the laboratory. Color and phenols were determined by spectrophotometric procedures described by S.P.R.I. (5). Other important non-sucrose constituents like glucose and fructose were also analyzed for each sugarcane juice by high performance liquid chromatography (HPLC) using a sugar analyzer (Waters Associates, Millipore Corp.) liquid chromatograph, cation exchange column in calcium form at 90°C, with aqueous 40 mM calcium acetate as mobile phase.

Effects of Cane Tops and Trash to Color on Sugarcane Juice

Commercial tops with green leaves as trash of MZC 74275 variety were collected on the field and additions from 2.5% up to 15.0% (weight basis) of well shredded tops to clean cane were executed in the laboratory. The sugarcane samples with different levels of tops were pressed in the hydraulic press and the sugarcane juices obtained were analyzed for color and phenols using the spectrophotometric methods published by S.P.R.I. (5).

RESULTS AND DISCUSSIONS

Gas Chromatography (GC) and Mass Spectral (MS) Identification of Major Compounds

The ethyl acetate extract (A) exhibited many peaks on GC-MS analysis when the sample was converted to TMS. The identification of compounds was based on the criteria of retention time and MS match. The automatic spectral search found 156 peaks on the ethyl acetate extract, 18 of which were the most important components (Table 1). The identified compounds were mostly phenolic acid derivatives, which originate from the cane plant and make up the colorant precursors that contribute to color formation in raw sugar.

Farber & Carpenter (3) in New Orleans identified syringic acid and caffeic acid in raw sugar, as well as p-hydroxybenzoic acid and 3-methoxy-4-hydroxybenzoic acid in cane leaf and ferulic acid and p-hydroxycinnamic acid in refined sugar. In Australia Paton (5) identified p-cinnamic acid, ferulic acid, caffeic acid, syringic acid, p-hydroxybenzoic acid, 3-methoxy-4-hydroxybenzoic acid and 3,4-hydroxybenzoic acid in raw sugar. The ethyl acetate (A) from a Colombian raw sugar contained all the above compounds reported by Farber & Carpenter (3), as well as glyceric acid and 3-hydroxybenzoic acid. Of particular interest was the presence of chlorogenic acid and two of its isomers. Another significant observation was the presence of a dimer of 5-hydroxymethylfurfural (HMF) which might be resulting from alkaline fructose degradation during the sugar processing.

Effects of Cane Burning on Color of Sugarcane Juice

The effect of temperature during cane burning on color formation in the sugarcane juice is shown in the Table 3. The data clearly demonstrated that there were significant differences in the color and an increase of 18.0% was found in the burnt cane with respect to the green cane. However, no significant differences of the phenols and total polysaccharides were detected between the burnt and unburnt cane. Nevertheless, increases of 9.8% and 2.7% respectively, were detected in this trial as a consequence of cane burning.

Effects of Cane Tops to Color on Sugarcane Juice

The addition of 1% of tops plus green leaves caused an increase of 2.7% in the color juice as well as higher levels of phenols in the juice were also observed with the increasing of this green trash (Table 4). This fact was also reported by Legendre in Louisiana and Lionett in South Africa (3), and it suggested the importance of harvesting clean cane in order to produce raw sugar of good quality.

CONCLUSIONS

The extraction method based on an enriched colorant fraction obtained by washing sugar crystals with methanol seems to be very effective and useful for further studies. The ethyl acetate extraction proved effective for extracting colorant precursors and permitted GC-MS analysis of the main components from raw sugar. GC analysis indicated the presence of several phenolic acids, chlorogenic acid, a dimer of 5-hydroxymethyl furfural (HMF), carbohydrate-related compounds and glyceric acid in the ethyl acetate extract.

The colorant compounds identified in the raw sugar were mostly phenolic acid derivatives, which are present in the cane plant.

Higher values of the color juices at pH 7.0 were found when the cane is burnt and the percent of trash, as green tops, increases in the harvested cane.

No significant differences in phenols were detected between burnt and unburnt cane.

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Table 1. Major colorant and pre-colorant compounds in the ethyl acetate extract of raw sugar from Colombia.

RT ¹ (min)	Compound	Area %
7.64	succinic acid	3.17
8.09	glyceric acid	0.70
12.64	3-hydroxybenzoic acid	0.89
13.84	p-hydroxybenzoic acid	1.87
17.40	3,4-dihydroxybenzoic acid	1.20
18.74	syringic acid	2.14
18.93	alpha-glucose	1.71
19.36	p-hydroxycinnamic acid	3.12
20.43	beta-glucose	1.14
21.65	ferulic acid	0.59
22.36	caffeic acid	0.43
24.94	HMF dimer	6.22
30.04	sucrose	1.91
44.45	chlorogenic acid	0.65

¹ RT : Retention time

Table 2. Major compounds in the methanolic-aqueous (9:1) extract.

Compounds	RT ¹ (min)
alpha-glucose	7.50
beta glucose	8.86
caffeic acid	11.71
sucrose	22.73

¹ RT : Retention time

Table 3. Effects of cane burning to color, phenols and total polysaccharides in sugarcane juice.

Treatment	Color ICUMSA (* 10 ³)	Phenols (mg/L)	Polysaccharides (mg/L)
Unburn	7.2 B ^{1/}	868 A	1871 A
Burn	8.5 A	953 A	1922 A
% Increase	18.0	9.8	2.7

^{1/} Means with the same letter are not significantly different (Tukey test).

Table 4. Effects of cane tops and leaves to color and phenols in sugarcane juice. Variety MZC 74275.

% Tops and leaves	Color ICUMSA (* 10 ³)	Phenols (mg/L)
0 % (Clean)	7.4	473
1 %	7.6	476
5 %	8.4	488
% Increase with 1 %	2.7	0.6
% Increase with 5 %	13.5	3.2

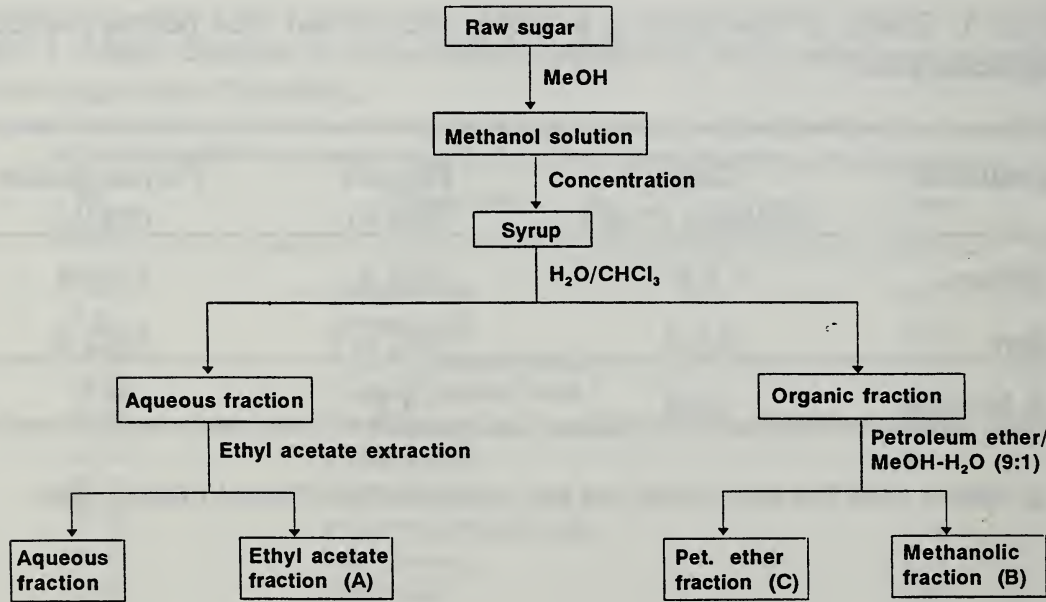


Figure 1. General isolation of colorants constituents from Colombian raw sugar.

DISCUSSION

Question: With green cane, do you have a problem with starch?

Larrahondo: We are fortunate in Colombia to have varieties that are no higher than 70 ppm in normal juice. When I compiled a survey of polysaccharides (and color) in Columbia (Proc. Int. Soc. Sugarcane Technol., 1989, v. 2:p. 591), we found that some varieties contain up to 120-130 ppm starch. Our Institute, Cenicaña, is responsible for new varieties in Colombia; we would not release a variety that had very high starch, even though it had high sucrose.

Question: You mentioned carbohydrate degradation products -polymers formed from carbohydrate - melanoidins, and alkaline degradation products. But in one slide you showed HMF as a precursor for the degradation product. In my opinion, HMF is formed under acidic conditions, so perhaps there are other acid degradation products.

Larrahondo: As you say, there are alkaline and acidic degradation products. In our raw sugar factories, the pH is generally acidic, but at the point of liming in the clarification vessels, there is a temporary very alkaline region before the lime is mixed well into the juice. So we can have both alkaline and acid degradation products.

THE ROLE OF SUGARBEET INVERTASE AND RELATED ENZYMES DURING GROWTH, STORAGE AND PROCESSING

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ABSTRACT

In a collaborative project between British Sugar plc and Sucros Ltd, four sucrose inverting enzyme activities were identified in sugarbeet; soluble acid invertase, insoluble acid invertase, soluble alkaline invertase and soluble sucrose synthetase. The activities of these enzymes were measured in the growing crop, during root storage and through the diffusion process.

Crop sample results confirmed many of the previous understandings on the phasing of plant growth and sucrose accumulation. It is likely that the enzymes active in sugarbeet before harvest are necessary in their entirety for healthy plant development.

During clamping and storage, a relationship between beet invertase and sucrose synthetase activities, and invert sugar was observed. Two distinct physiological pathways influencing these activities are proposed. Clamping and storage trials were performed both in U.K. and Finland under very different climatological conditions. Typical late autumn and early winter freezing and thawing in Finland caused significant invertase activity increase in beet starting to deteriorate. Microbial invertases analyzed showed significantly higher thermostability compared to beet invertases.

Results from healthy beet process sampling indicated that only a small fraction of the difference between sugarbeet and raw juice invert sugar concentration was due to sugarbeet derived invertase enzymes. While processing frozen and thawed beet material, the invert sugar difference was higher, but still only partially explained by elevated invertase enzyme activities in the beet material. The results emphasized the importance of rapid temperature increase in diffusion.

It is possible to estimate the considerable financial impact of invert sugar formation during clamping and processing. This report provides information useful in the reduction of this cost.

INTRODUCTION

Four enzymes capable of inverting sucrose have been detected in sugarbeet roots:

cell wall acid invertase,
vacuolar acid invertase,
alkaline invertase and
sucrose synthetase.

Although their roles in sucrose synthesis, translocation or storage are not fully understood, it is generally agreed that during the first year of growth, the function of invertase is primarily to support maintenance metabolism and cell expansion. During the second year of growth, invertase hydrolyses sucrose stored in the vacuole, producing invert sugars for the metabolism of the plant and ultimately seed production.

A consequence of this cycle of activities is that when the sugarbeet is harvested at the end of the first year of growth, the sucrose content of the beet is at or near its maximum, but the root has the full compliment of sucrose inverting enzymes or the potential to produce them.

During the latter stages of growth, storage and in the initial parts of the sugar extraction process in the factory, these enzymes can be activated leading to a decrease in the sucrose content of the beet and an increase in the level of invert sugars. The implications in terms of extraction losses and increased molasses formation are large.

This paper reports the results of experimental work covering sugarbeet during growth, storage and processing, both in Finland and U.K. Conclusions of this work can be used to estimate the financial impact of these enzymes on sugarbeet processors and provides information useful the reduction of this cost.

INVERTASE ACTIVITIES IN THE GROWING ROOT

Before describing the profiles of the individual enzymes through the growth cycle, it is useful to review the location of the enzyme systems within the typical beet storage cell (Figure 1).

Within the typical beet storage (parenchyma) cell, there are two distinct environments, the cytosol and the vacuole, differing mainly in their pH. The vacuole is acidic (pH 4.5-5.0), whilst the cytosol is mildly alkaline (pH 7.5-8.0). In sugarbeet, sucrose is stored in the vacuoles of these cells (17). The concentration of sucrose in the vacuole is 0.7 to 0.8M. This concentration is a hundred times greater than in the cytosol (13). This difference across the tonoplast (vacuole membrane) is created and maintained by a proton/sucrose transport mechanism (1) which may involve potassium ions as well (13). Invertase plays no role in the uptake of sucrose into the vacuole of the root storage cells (1,2,4-6,11,13).

In young, rapidly growing and developing sugarbeet, there are high levels of acid invertase (and consequently high levels of glucose and fructose) (14). Levels gradually fall over time. After 40 to 45 days of growth, acid invertase activity is very low and sucrose accumulation intensifies (2,7). Whether this reduction of activity is due to the absence of enzyme (7) or the presence of an endogenous inhibitor (8) is unclear. It is likely to be a mixture of the two.

Soluble acid invertase regulates cell expansion, and hence root growth. Sucrose is transported into the vacuole of the growing cell. Here it is hydrolyzed by the high levels of acid invertase activity to produce glucose and fructose. This increases the osmotic pressure of the vacuole, sucking water into the cell. The resulting increase in turgor pressure forces the cell to expand.

As the root matures, acid invertase activity declines, permitting sucrose accumulation in the storage cells (2).

The insoluble acid invertase is located in the cell wall, and is reported to hydrolyze sucrose across the cell wall (3).

The alkaline enzymes, soluble alkaline invertase and sucrose synthetase, are located in the cytosol. The profiles of these enzymes during growth are essentially identical,

and they are reported to perform similar roles. Consequently, they can be discussed together.

The data of Masuda (9), shows the enzyme profile during establishment, growth and storage for alkaline invertase. Whilst cytosolic enzyme activity is very low during root establishment, this begins to increase after about 35 days and reaches a maximum after about 120 days. This then falls to 25% of peak activity and remains at this lower level during storage. The reducing sugars or UDP-glucose produced are used for maintenance metabolism (3).

Our experimental results have confirmed many of these reports.

Our studies encompassed three common varieties, Saxon (Hilleshog) and Cordelia (KWS) in U.K., and Univers in Finland, across four plots in each country. Sampling began 60 days after sowing in Finland and 80 days after sowing in U.K., and was continued on a regular basis until harvest.

The data in Figures 2 & 3 shows the acid soluble and insoluble invertase activities for beet sampled throughout the growing period. The profiles during growth are essentially identical. These results suggest that the acid invertase activities were falling from a higher level established before sampling commenced, as discussed above. By 75 days, both soluble and insoluble acid invertase activities appeared to have settled to a level consistently below 5 nmol sucrose/min/g beet, having been at 40-50 and 15-25 nmol sucrose/min/g beet respectively early in the sampling period.

The high levels of acid invertase activities in the early stage of growth are associated with rapid plant growth, low sucrose concentration and high invert sugar concentration (Figures 4, 5 & 6). As the levels of acid invertase activities fall, so plant growth slows, sucrose concentration rises and invert sugar concentration with respect to sucrose falls. At harvest (150 days growth in Finland, 220 days in U.K.), the very low levels of acid invertase activities are accompanied by maximum plant weight, maximum sucrose concentration and minimum invert sugar concentration.

As reviewed above, high soluble acid invertase activity is required for cell expansion and root growth. The fall in soluble acid invertase activity allows sucrose to be accumulated in the vacuole. Clearly, insoluble acid invertase follows a similar pattern. As the cell is expanding this enzyme assists in increasing the invert sugar

concentration, causing the cell to swell. As the plant matures, the insoluble acid invertase activity falls to allow sucrose accumulation.

Figures 7 & 8 respectively show the activities of soluble alkali invertase and sucrose synthetase throughout the growing period. A profile similar to that reported by Masuda (9) was observed. Between 60 and 140 days growth, both activities fell from a maximum to a lower value (20 and 30 nmol sucrose/min/g beet respectively). Between 140 days and harvest, these activity levels were maintained. Throughout the growth period studied, these enzymes remained the major sucrose inverting activities present in the root.

The role of these enzymes in maintenance metabolism has been reviewed above. Studies on invertase activities during clamping reported below will also show a role for these cytosolic enzymes in respiration. To enable rapid plant growth, high levels of respiration and metabolism are required. As the plant matures, these levels will fall. At harvest, the plant is preparing for winter; respiration and maintenance metabolism will be the minimum necessary to keep the plant alive.

INVERTASE ACTIVITIES DURING CLAMPING IN THE U.K.

In the U.K., a 1000t clamp was built in early December 1993 and broken up sequentially after 12, 35, 50, 65 and 80 days. The clamp had walls of straw bales on pallets and was covered by plastic sheeting. For each sample day, a net containing ten beet was placed at positions 1,3,4 and 7 in the clamp (see Figure 9). These positions were a representative selection of all of the positions in the clamp. As the clamp was built (day 0), four separate samples of ten beet were taken for immediate analysis. The beet going into the clamp were quite dirty and wet. The clamp at 65 and 80 days was very wet, with many beet sprouting.

No trends between the different positions in the clamp were observed. Mean activities over the four positions are shown in Figure 10.

Throughout the trial, soluble alkali invertase and sucrose synthetase were the major activities present. However, during the trial, their proportion of the total activity decreased from 93.1% to 58.2%. This was due to both a decrease in these cytosolic activities and an increase in the acid invertase activities.

The high acid activities for 80 days were due to position 3, which was located at the bottom of the middle of the clamp (29.5 and 23.8 nmol sucrose/min/g f.w. respectively) (table 1). Acid activities in the other positions remained essentially the same between 65 and 80 days.

The factors that influence the rate at which roots respire are, in order of importance: (15) temperature, relative humidity of the air, oxygen and carbon dioxide content of the air and the presence of microbial substances. Because the roots cool after being harvested the rate of respiration falls. Consequently, the invertase activities required to hydrolyze sucrose to fuel respiration fall.

A decrease in both soluble alkali invertase and sucrose synthetase activities over the first 50 days after harvest was observed. This was associated with a fall in the rate at which invert sugar concentration increased. Figure 11 shows the sum of alkali invertase and sucrose synthetase activities, the sum of soluble and insoluble acid activities, the sum of all activities, the invert sugar concentration and the rate of invert sugar concentration increase during the trial. The 'field' sample represents the final crop samples taken from the U.K. plots at the end of October. This is shown for comparison. After 65 days there is only a slight increase in alkali invertase and sucrose synthetase activities. However, during this time there is a dramatic rise in the rate of invert formation.

After harvest, there was an increase in soluble and insoluble acid invertase activities. There was then little change until 50 days of storage. After 50 days there was a further increase in soluble and insoluble acid invertase activities, accompanied by an increase in invert sugar concentration.

Similar behavior of invertase activities in beet during storage has been reported by other workers (16). In their study, beet were harvested and then stored at three different temperatures (2, 5, and 21°C) for different lengths of time. Under all storage conditions, the activity of alkali invertase and sucrose synthetase fell by 50 to 60%. Soluble acid invertase activity was closely related to invert sugar concentration. Soluble acid invertase activity was barely detectable at harvest, but over 30 days increased 10-fold at 2°C and 28-fold at 21°C. This was associated with rates of invert sugar concentration increase of 0 and 3.6 mg/100g f.w./day at 2 and 21°C respectively.

We are able to propose a model of the processes in the stored sugarbeet root influencing invert sugar formation (Figure 12). Soluble alkali invertase and sucrose synthetase are present in the cytosol. Respiration occurs in the mitochondria in the cytosol. Consequently it is likely that both soluble alkali invertase and sucrose synthetase are responsible for the hydrolysis of sucrose to fuel respiration. The root is unlikely to hydrolyze sucrose to invert that is not required. The activities of soluble alkali invertase and sucrose synthetase are, therefore, likely to be governed by the rate of respiration. The factors that govern the rate of respiration have been mentioned above.

Evidence supporting this relationship between storage temperature and cytosolic activities is also presented in Figure 13. Samples of beet were stored at different temperatures (0, 4, 10, & 15°C) for 80 days. The activities of the four invertases identified were measured after this time. There is significantly greater cytosolic activity in the 15°C experiment than in the cooler temperatures.

In the growing plant the rate of respiration is high, fueled by high soluble alkali invertase and sucrose synthetase activities. However, the invert sugar content is relatively low; the plant is only producing invert sugar at the rate at which it is consumed by respiration and other metabolic functions.

After harvesting, the roots cool in the air or in the clamp. Therefore the rate of respiration decreases, causing a drop in soluble alkali invertase and sucrose synthetase activities.

Sucrose and soluble acid invertase are both present in the vacuole (8). Insoluble acid invertase is associated with the cell wall (10). In the 12 days after harvest an increase in invert sugar concentration and soluble and insoluble acid invertase activities was observed. It is proposed that the increase in the acid activities is caused partly by the halt of normal growing conditions and partly by the wound response mechanism of the root following topping and harvesting. This increase may be responsible for the initial increase in invert sugar concentration. After the initial shock of harvesting and clamp building the roots were not stressed further until after 50 days. Between 12 and 50 days the acid invertase activities fell slightly, lowering the rate of invert sugar formation. During sampling at 65 and 80 days, the clamp was visibly wetter than before and many roots had begun to sprout. Acid invertases are reported to be active in areas of root growth (12). They are therefore likely to be active in sprouting roots.

It is proposed that the activity of acid invertases is controlled by regrowth (sprouting), which requires invert sugar for cell expansion, and stresses such as wounding. The rise in the rate of invert sugar concentration increase after 65 days is caused by this increase in acid invertase activities since there is little increase in the alkali invertase and sucrose synthetase activities during this period.

There were no significant differences in root properties and enzyme activities between the positions in the clamp except for position 3 after 80 days. Table 1 shows the individual values for position 3 and the means of the other positions for invert and enzyme activities.

Position 3 after 80 days had a particularly high invert sugar concentration. This was accompanied by an increase in soluble and insoluble acid invertase activities. Position 3 was at the middle of the bottom of the clamp (Figure 9). This position in particular was very wet and a lot of sprouting was evident. Results from this position are the basis of the hypothesis that the increase in invert after 50 days during this trial was caused by acid invertase activities rather than soluble alkali invertase or sucrose synthetase activities

INVERTASE ACTIVITIES DURING CLAMPING IN FINLAND

The cultivation region of sugarbeet in Finland is between 60° and 62°N, which is the same latitude as Anchorage or south Greenland. Due to the warming effect of Gulf stream, it is possible to grow beet in Finland. Finland's geographical location and climate puts a heavy burden on cultivation, harvesting and clamping compared to other regions of Europe. Finnish beet factories start their campaign during the last week of September and normally the campaign is finished before the 2nd week of December (about 70 days campaign).

Finnish clamps typically contain 50-100t beet, depending on the harvesters and farmers' cultivation area. The clamp must be protected with a canvas or some other cover against the first serious frost (-10°C for a day or more), normally occurring during the 1st half of November. In the second half of November there are occasional frosts between warmer periods. During December the average temperature drops permanently below zero. Figures 14 A and B show the average daily minimum and maximum temperature over the last 30 years (1966-1996), together with the air temperatures (1 m above the surface) measured during the clamping 1994 and 1995.

The objective of Sucros Ltd. for the clamping trials was to find practical means to extend the normal campaign to 100 days, *i.e.* to mid-January. The reduction of sugar loss through enzymatic inversion will be essential to achieve this target. Enzymatic inversion during clamping can be caused by biocatalyst both from original beet and by contaminating microbes.

The trial clamps containing 20 - 50t beet were built during late October. The bottom of the clamps were surrounded with a row of straw bales, and a layer of peat spread over the surface of the clamp to avoid frost damage. Peat addition was governed by clamp temperature to avoid freezing or excess warming.

1994 samples were manually harvested and topped. Both topped and untopped samples were used. Several nets, each containing 20 beet, were placed 80cm deep on the side of the clamp. Because the clamping trial 1994 was extended to 160 days, machine harvested beet close to the site of the nets were used for the later samples (clamp beet). In 1995, only machine harvested beet from 80cm deep were used for assays. Throughout both trials, the clamps appeared dry and the roots remained in good condition.

Reference samples were collected from the surface of an uncovered, small beet piles besides the test clamp. The reference samples thus represent the worst storage scenario. A short time after the first frost, the appearance of the reference sample started to change. The cut surface became first transparent and then semi-opaque, the beet tissue became soft and emitted odors of fermentation. Considerable microbiological growth was obvious.

During the trial 1994, the total dry matter of the roots decreased slowly. The initial dry matter was 22.0% and during 100 days clamping it decreased linearly to 20.8%. After 150 days, total dry matter was 20.2%. The total dry matter of the unprotected reference sample decreased rapidly. The rate of dry matter loss was 0.12% per day, 10-fold that of the samples in the clamp.

The marc content of the beet in the clamp did not change significantly during the trial. No difference between the dry matter and the marc of topped and untopped samples was detected. The total dry matter and the marc contents of the samples are given in Figure 15.

The sucrose content of the samples decreased gradually from 15.35% (pol) to 14.41% within 100 days, and further to 13.94% within 150 days (1994). Both the sucrose and the total dry matter loss went hand in hand. It is most likely that the observed sucrose loss was due to the use of sucrose to fuel vital metabolic functions of the cell and the sprouting. The results from the trial 1995 showed a lower sucrose loss rate due to the lower clamp temperature. The sucrose content of the topped beet was slightly higher compared to the untopped, but the sucrose loss rates were similar. The unprotected reference sample lost sucrose at a very rapid rate. Within 40 days no sucrose could be detected. The rate was 40-fold that of beet in the clamp. The results of standard pol assays are given in Figure 16. Samples that could not be filtered for pol were assayed by HPLC.

The invert sugar content increased linearly in the samples. The initial invert value was 0.072% of the beet (0.47% S) in 1994. After 100 days clamping invert content doubled to 0.14% of the beet (0.98% S) giving daily increase of 0.005% S. Extending the clamping further, the increase rate remained the same. No difference between topped and untopped beet samples could be detected.

The invert sugar content of the unprotected reference samples increased rapidly when the microbiological deterioration started, but plateaued at 2.5% fresh weight. This maximum invert value can be explained by rapid microbiological activity throughout the whole of the root. Any invert sugar produced was promptly utilized for both microbial cell mass production and polysaccharide synthesis. It is well known that dextran, levan and other polysaccharide concentrations increase rapidly in spoiling roots.

The microbiological contamination throughout stored roots was measured using aseptic sampling technique and plating methods. Surprisingly high microbial counts were detected from healthy looking roots. The microbes had evidently penetrated the root through xylem vessels after topping. In the body of the root, microbial counts were high and uniform; close to the skin, lower counts were measured. Close to the skin, the vessel structure is poorly developed, preventing perhaps microbe penetration. Pure cultures of the species isolated were tested. Most of them produced polysaccharides and microbial invertase. Figure 17 shows the counts through the cross section of two healthy looking roots (from clamp in December 1994).

When the beet freeze slowly during a prolonged frost, large ice crystals are formed. These crystals break the plant cell structure. When the plant tissue thaws, all microbes

present inside the root are able to metabolize sucrose released from ruptured vacuoles. Although many microbes can metabolize sucrose, invert sugars are preferred. The presence of sucrose induced invertase enzyme synthesis in most of the microbe species plated from the root tissue. Each species produced invertase activity having slightly different thermostability and pH optima. The microbial invertases were more thermostable than sugarbeet root invertases and had a pH-optimum of approximately pH 6.

It is common to find the first filtration problems in the factory about two weeks from the first frost and thaw. Filtration problems are mainly caused by polysaccharides.

Throughout the trials, invertase activities in the clamped samples remained at low levels. Sucrose synthetase and alkaline invertases were the main activities, insoluble acid invertase activity was low and soluble acid invertase activity almost zero. During 1994, the activity of alkaline invertase increased linearly from an initial value of 18 to 28 nmol/min/g within 100 days. The activity of sucrose synthetase remained unchanged through the first 100 days (50 nmol/min/g), but toward the end of clamping cycle it started to increase. Both acid invertases were initially at low level. The activity of insoluble acid invertase increased slightly, while the soluble acid invertase activity remained unchanged.

The total invertase activity of a healthy beet at the beginning of clamping was 87 nmol/min/g beet, consisting of sucrose synthetase (57%), alkaline invertase (19%), insoluble acid invertase (16%) and soluble acid invertase (7%). After 100 days clamping the total activity increased to 104 nmol/min/g and consisted of slightly increased sucrose synthetase (53%), increased alkaline invertase (27%) and virtually constant acid invertase (14% and 6% respectively). No significant differences in invertase or sucrose synthetase activities between topped and untopped roots were observed. Figures 18, 19, 20 and 21 show the measured invertase activities.

Results from the unprotected reference samples were dramatically different. Alkaline invertase activity increased to a maximum of 360 nmol/min/g. Soluble acid invertase activity peaked at 1000 nmol/min/g and insoluble acid invertase activity slightly lower at 310 nmol/min/g. Sucrose synthetase assay gave negative values, probably due to microbes producing components which interfered with the sucrose synthetase assay. These activity increases went hand in hand with observed microbiological deterioration. The assayed activities are shown in Figure 22.

Why is the picture in reference sample so different? The reason may lay behind the microbiological contamination and the properties of the multitude of microbiological invertase induced into the system.

The routine enzyme activity assay used in this work was unable to distinguish microbial invertase activities from endogenous beet invertase activities. Only sophisticated immunochemical assays lying outside the scope of this work would be able to make the distinction.

Enzyme catalysis is usually controlled by diffusion. As the temperature in the reaction mixture increases, diffusion is improved and the expression of the activity of each enzyme is increased. Typically each 10°C increase doubles the rate of invert sugar production. However, the enzyme, being a protein molecule, is susceptible to thermal denaturation; the higher the temperature, the quicker the denaturation. Proteins derived from different sources show very different abilities to tolerate higher temperatures. Usually plant enzymes have lower thermostability compared to microbial enzymes.

The pH optimum and thermostability of invertase in two different test samples were assayed. The first sample, a growing beet at the beginning of September 1993, could be assumed to contain only endogenous beet enzymes. The second sample was an unprotected reference beet sampled in early November 1993 which had just begun to spoil. The activities of alkaline invertase, sucrose synthetase, soluble acid invertase and insoluble acid invertase in the second sample were 38.8, 52.1, 19.2 and 23.0 nmol/min/g respectively. The activities measured from a one week older healthy clamp sample were 14.8, 42.8, 3.7 and 4.5 nmol/min/g respectively.

Figure 23 shows the relative activities (in % of the maximum) measured in two different buffer systems covering the pH range from 3 to 9 (0.1 M citrate and glycylglycine buffer). The figure demonstrates, that significant amount of invertase activity in neutral and acid pH's has developed when spoiling is still at an early stage. This activity range is relevant to the process, because the pH in diffusion is typically 5.7, consequently. These new microbial invertases can express their full potential in the factory diffusion.

The thermostability of the two samples were tested by keeping them in a weak buffer solution at pH 6.0 in a water bath. After treatment in the bath the samples were promptly cooled. The invertase activities were assayed using normal assay procedure.

Figure 24 shows the relative activities of alkaline invertase assayed from the samples after heat treatment. The figure clearly demonstrates how spoiling increased the thermostability of invertase activity. Over 50% of the pure plant enzyme is inactivated within less than three minutes. The spoiling sample contained both plant enzyme and microbial enzyme. The plant enzyme was rapidly inactivated, but the more thermostable microbial enzyme survived at the elevated temperature for much longer. Figure 25 shows the same phenomena with insoluble acid invertase in the spoiling sample. (The insoluble acid invertase activity in the growing beet was so low, that it could not be used as a reference in Figure 25.)

The complex combination of invertase activities in sugarbeet, originating both from the plant itself and from microbial origin, in the early stage of spoiling makes enzyme activity data difficult to interpret. This could be the reason for the differences noted in the tests made in Finland and in the U.K. The clamp in Finland could have been less contaminated with microbes than the clamp in the U.K., which was reported to be wet. The spoiling unprotected reference roots outside the clamp in Finland were obviously more contaminated.

INVERTASE ACTIVITIES DURING PROCESSING

Finnish beet sugar factories use a prescalder-tower diffusion system. The slicing capacity of each of each factory is approximately 250t/h.

The quality of beet processed during 1994 and 1995 was good. A sieved fraction containing beet tails was washed separately, grinded with a hammer mill and taken with the wet pulp stream coming from the tower into the pressing station. Press water from the pulp presses and water from the tail fraction was collected into a tank. The press water tank had a residence time of between 10 and 15min before the water was pumped through a heat exchanger to the extraction tower. There was a sampling valve in the pump line. Condensate from evaporators was used as make-up water to a draft of about 112%. Raw juice drawn from the cold end of the prescalder was pumped directly into the cold preliming. There was only a short residence time from the prescalder to the point when the pH was elevated. The pH increase inactivated the invertase activity promptly.

The temperature-residence time profile of the cossettes in the diffusion unit in Salo factory is shown in Figure 26. Ideal mixing in the prescalder and in the tower was assumed. The typical temperature target in the tower was 71°C. The prescalder

worked efficiently, increasing the temperature of the mixture to over 60°C within 10 minutes.

Diffusion unit sampling was possible through four valves in the prescalder, two in the tower together with single samples of cossettes, the raw juice to preliming line, the recycling juice coming back from the tower, wet pulp, pressed pulp, press water and condensate.

The cossette and juice mixture drawn from any sample valve was immediately separated into liquid and solid fractions by pouring through a normal kitchen sieve. The solid fraction was briefly flushed with cold water to remove excess juice and to cool the sample, then promptly ground coarsely. Samples of both juice and solid were frozen in dry ice. A complete sampling cycle was performed three times, with two hours interval on each sampling day. Samples from each cycle were averaged after properly mixing them for assay.

Invertase activities of fresh cossettes were low. No filtration problems towards the end of both campaigns occurred. Invertase activities were measured in both liquor and solid samples.

The four invertase activities assayed in fresh cossettes through the campaign 1994 are shown in Figure 27. In raw juice drawn from the prescalder, all the invertase activities were less than 10 nmol/min/g solution (Figure 28). When examining the invertase activity decrease along the prescalder, it is obvious that the invertase activities are inactivated before the cossettes reach the tower. Figures 29, 30 and 31 show the activities measured for the alkaline invertase, both in cossettes and in solution, and the soluble acid invertase activity in cossettes. In these figures sampling points 1, 2, 3 and 4 are fresh cossettes, the prescalder cold end, the mid-section and the hot end respectively. The effective time for the enzymes assayed to express their activity is limited to a maximum of 15min before thermal denaturation is complete.

Samples taken from the recycle juice from the tower, from the wet pulp, and from the pressed pulp had no measurable invertase activity. Surprisingly, no activity was found in the press water pumped into the tower, even when it contained press water from tails. The sampling valve was after the press water collection tank. Any activity in the liquor pressed from tails is obviously thermally inactivated during the retention time in the tank.

The change of conditions through the prescalder from the cold end towards the hot end is very dynamic from the enzyme point of view. Invertase entering the system in the fresh cossette diffuses into the liquid together with the sucrose. Simultaneously, the temperature of both cossette and the solution rise, denaturing the enzymes. While inside the cossette (either in the beet cell or in the microbial cell), the enzymes usually benefit from some chemical and biochemical protection, inhibiting the rate of inactivation. After the release into solution, the protection is lost, and thermal inactivation is then imminent, depending on the protein structure. As discussed above, the more thermostable microbiological invertases will survive longer than beet invertases.

Invertase activities in this work was normally assayed at 37°C. One unit of activity expressed from one gram of beet sample is able to hydrolyze one nmol of sucrose within one minute. By definition a beet sample containing 100 units of total invertase activity can hydrolyze during 15 min incubation at constant +37 °C temperature about 0.5 mg sucrose. Typically in diffusion 1 g beet produce 1.1 g raw juice having 14.5 Pol and 0.6% invert on sucrose. Enzymatic invert sugar produced theoretically in the prescalder explain about 40% of invert assayed from raw juice. If the total invertase activity is much higher as found in seriously spoiled beet, e.g. 1500 units, the sucrose loss increases 15-fold and are up to 6 % invert on sucrose. This figure is most likely an under estimate of the inversion potential, because the enzymes present in spoiled beet are active longer at elevated temperature than the ones assayed during this test.

The dynamic situation in the prescalder makes it difficult to build a mathematical model to calculate the effect of enzymatic inversion. However, it is most likely a rather good estimate to take 37°C as the average temperature through the prescalder. The effects of activity increase and denaturation rate with increasing temperature will combine during the 15 minutes in the prescalder. To reduce sugar loss when processing spoiled beet, the prescalder temperature should be set higher. The value calculated using 37°C indicates that both in Finland and the U.K., than from one third to half of the invert sugar increase between cossettes and raw juice is due to invertase activity.

The effect of seriously spoiled beet material on the degree of enzymatic inversion is significant. The harmful effect of spoiled fraction is often diluted by the flow of good quality beet, and could be easily neglected. However, spoiled beet cause other problems during processing due to their high polysaccharide content. Strict quality control of the amount of spoiled beets entering the process is therefore required.

CONCLUSIONS

- * Four sucrose inverting activities were identified:
 - cell wall (insoluble) acid invertase,
 - vacuolar (soluble) acid invertase,
 - cytosolic (soluble) alkali invertase,
 - cytosolic sucrose synthetase.
- * High levels of invertase activities and invert sugar concentrations were observed in young, developing roots. In maturing roots, invertase activities fell, allowing sucrose accumulation. At harvest, invertase activities and invert sugar concentrations had fallen to a minimum; sucrose concentration had risen to a maximum.
- * There was an increase in invert sugar concentration during clamping. This was accompanied by a fall in total invertase activity, but a rise in acid invertase activity. These results indicate a causal link between acid invertase activity and invert sugar concentration during clamping. The clamping trials in both U.K. and Finland emphasize the need for good clamp management, especially keeping the temperature as low as possible while avoiding frost damage.
- * In spoiling beet, there was a dramatic rise in all invertase activities and the rate of sugar loss. Studies demonstrated that this was due to microbial invertases, which were more thermostable than the endogenous beet enzymes.
- * In a prescalder / tower diffuser process, beet invertases were quickly denatured within the prescalder. Consequently, these enzymes accounted for less than one third of the difference in invert sugar concentration between cosettes and raw juice. Microbial invertase activities entering the process in rotten or damaged beet were capable of dramatically increasing sucrose inversion during processing. This demonstrates the need to reduce to a minimum the amount of rotten beet entering the factory.

BASIS OF ASSAY METHOD

During the field sampling, the roots were weighed clean with and without leaves at the side of the field before transportation to the laboratory under ice.

The roots were washed by hand under cold running water and dried with a tissue. They were then sterilized by submerging in ice-cold dilute formaldehyde solution for 10 minutes, rinsed under cold demineralized water and dried with a tissue.

The roots were then topped and grated. Samples were taken for sucrose, glucose and fructose analysis and dry matter determination.

A sample of the remaining gratings was suspended in an extraction buffer (2-mercaptoethanol (5mM), EDTA sodium salt (1mM), phenylmethylsulphonyl (0.5mM) and sodium phosphate adjusted to pH 7.2 (100mM)) and degassed. The resulting suspension was homogenized.

The homogenate was filtered and the filtrate centrifuged. The resulting supernatant was dialyzed overnight to remove the sugars. The filter cake was repeatedly resuspended and filtered. Two samples of the washed cake were taken for dry matter determination; the remainder was stored in a sealed container at 4°C overnight.

The soluble protein content of a sample of dialysate was determined using a Bio-Rad kit. Aliquots of the dialysate were incubated in sucrose containing buffers at pH 4.5, pH 7.2 and pH 7.2 with UDP to assay for soluble acid invertase, soluble alkali invertase and sucrose synthetase respectively. Samples of the cake were incubated in a sucrose containing buffer at pH 4.5 to assay for insoluble acid invertase. The invert produced in these incubation mixtures was determined using the DNS (3,5-dinitro salicylic acid) method.

The activities of each enzyme were expressed as nmol sucrose / minute / g fresh weight, and the soluble enzymes as nmol sucrose / minute / mg total soluble protein. Analysis for sucrose, glucose and fructose was performed using a Boehringer-Mannheim kit. 26.0g of gratings were macerated for 3 minutes with 178.2g of 0.2% $\text{Al}_2(\text{SO}_4)_3$. The macerate was then filtered through a 0.45 μm membrane. The filtrate was used neat for glucose and fructose determination, but diluted by 20 for sucrose determination.

ACKNOWLEDGMENTS

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Table 1. Comparison of position 3 with other positions after 65 and 80 days.

Day	65		80		
Position	1,4 & 7	3	1,4 & 7	3	
invert	1.08	1.19	1.10	3.78	g/100gS
soluble acid invertase	11.2	8.9	8.7	29.5	nmol S / min / g f.w.
insol. acid invertase	14.5	11.0	12.4	23.8	nmol S / min / g f.w.
sol. alkaline invertase	16.4	16.3	17.1	21.4	nmol S / min / g f.w.
sucrose synthetase	20.5	24.8	23.9	18.3	nmol S / min / g f.w.

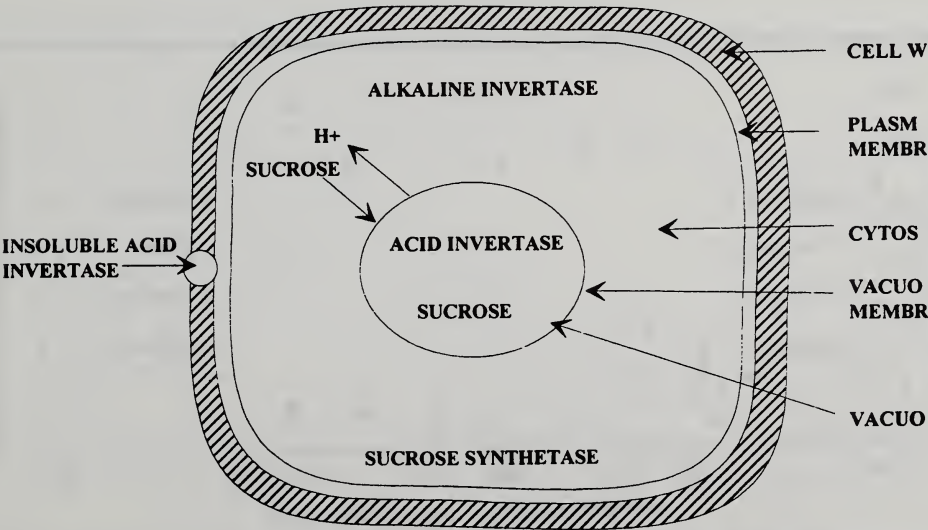


Figure 1. A typical sugarbeet root storage cell.

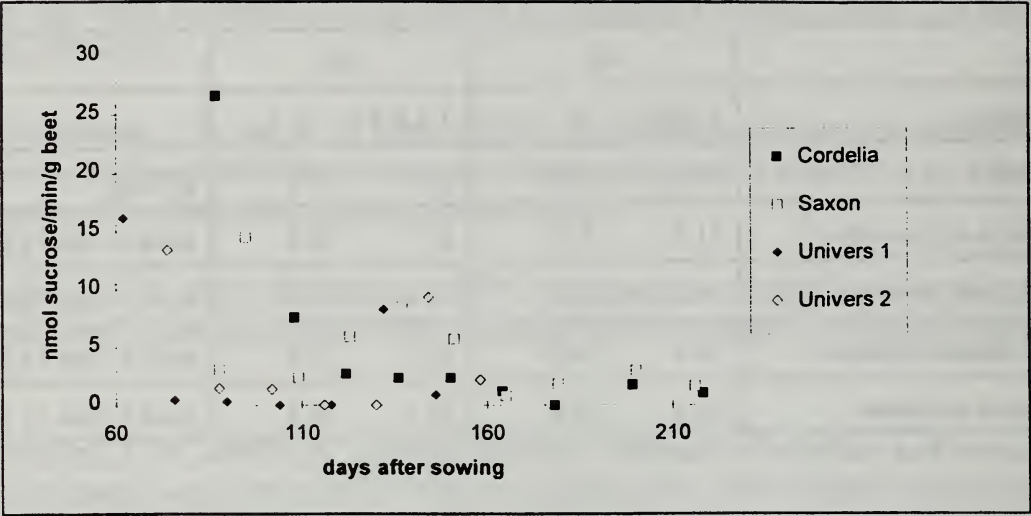


Figure 2. Soluble acid invertase activity during growth.

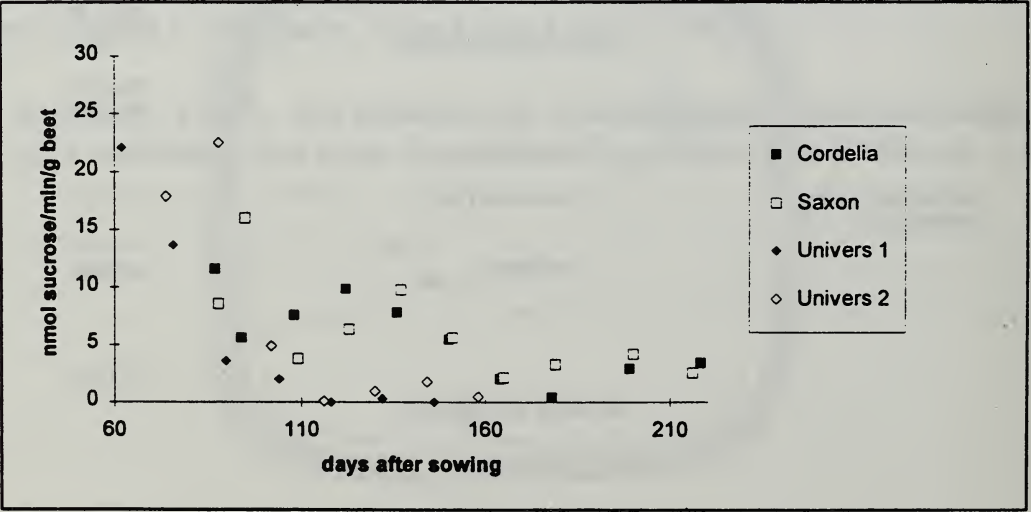


Figure 3. Insoluble acid invertase activity during growth.

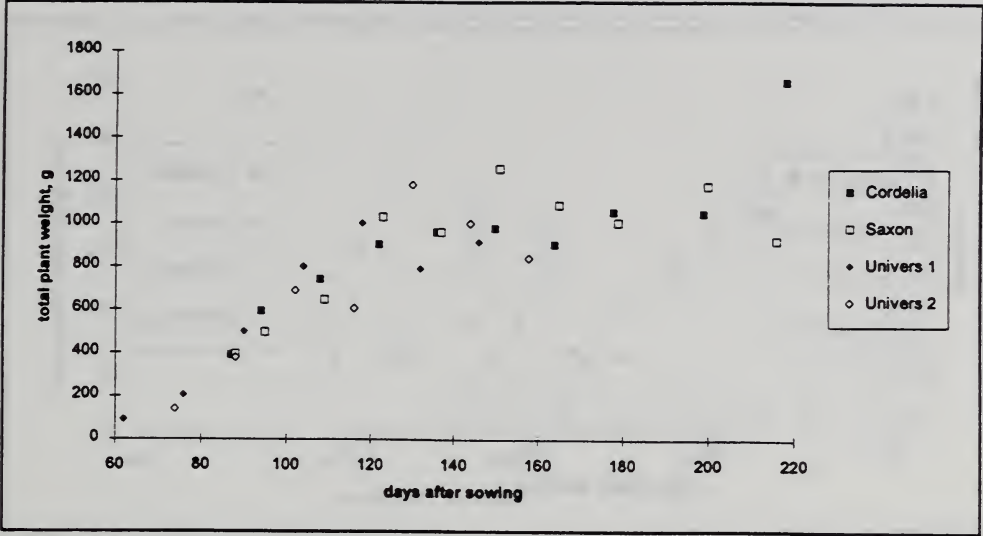


Figure 4. Total plant weight during growth.

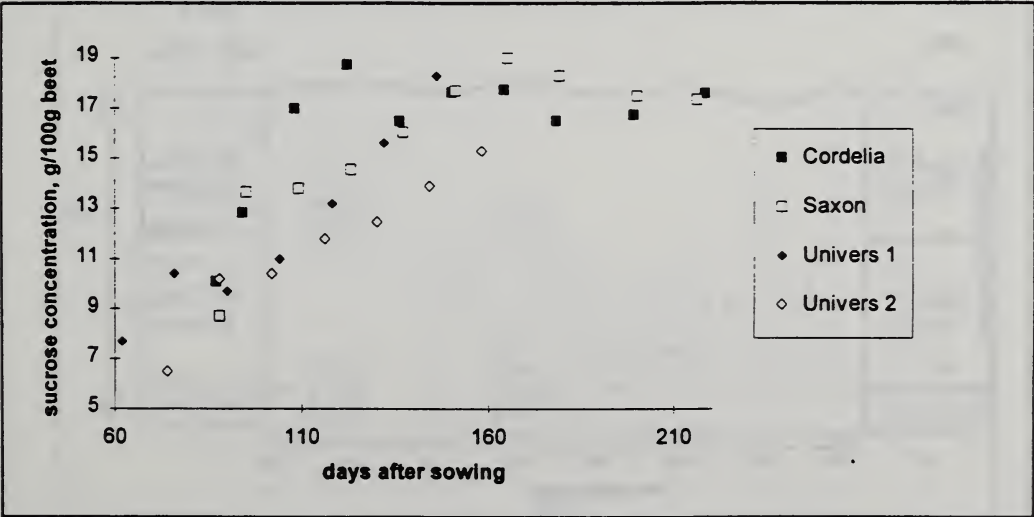


Figure 5. Sucrose concentration during growth.

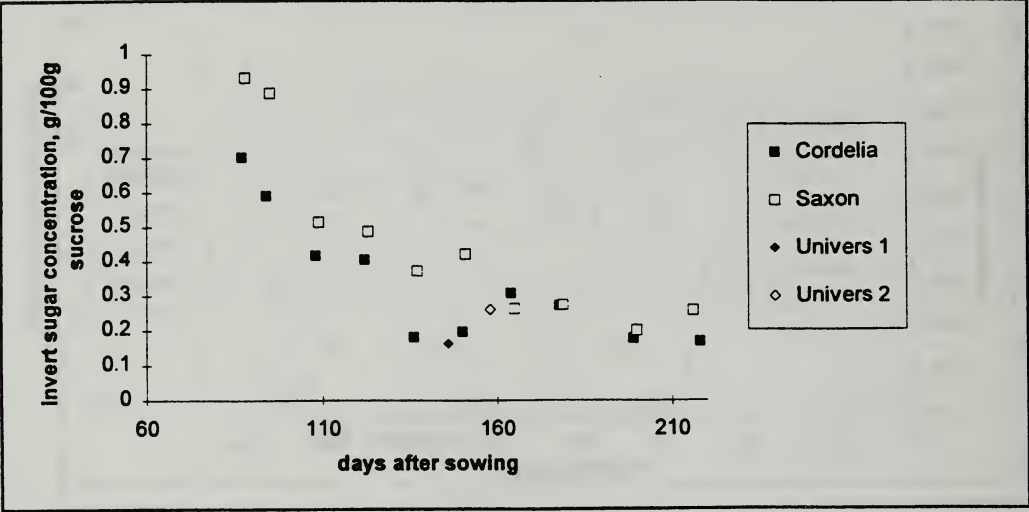


Figure 6. Invert sugar concentration during growth.

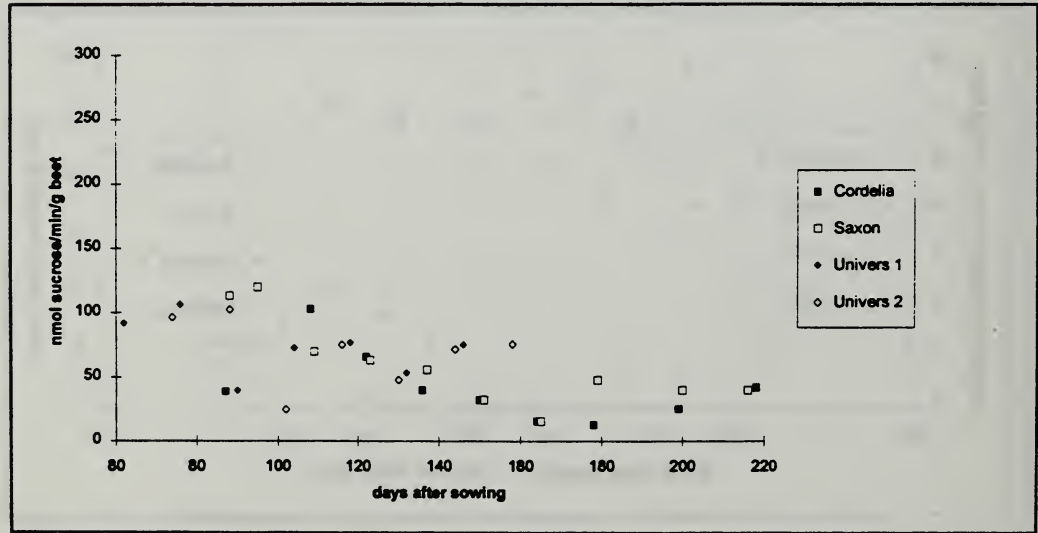


Figure 7. Soluble alkali invertase activity during growth.

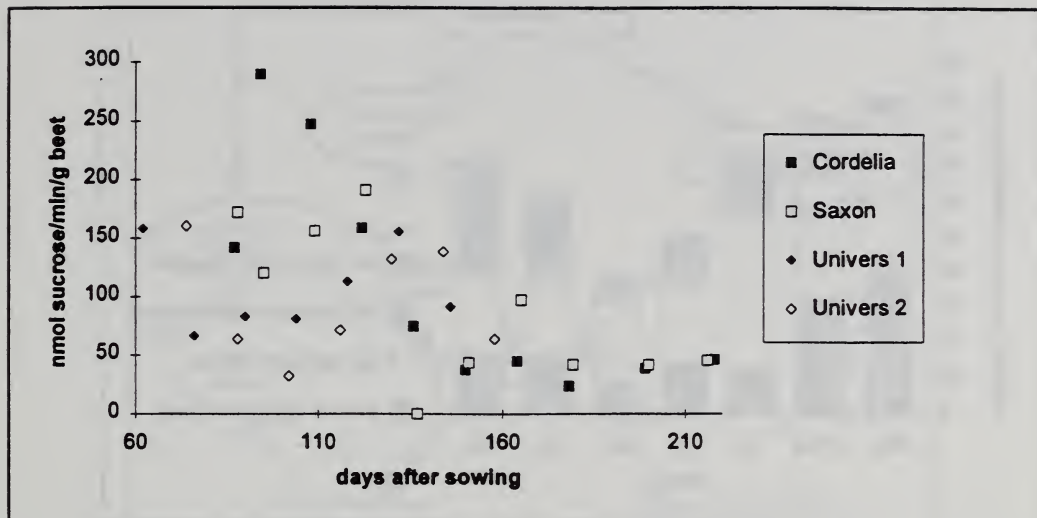


Figure 8. Sucrose synthetase activity during growth.

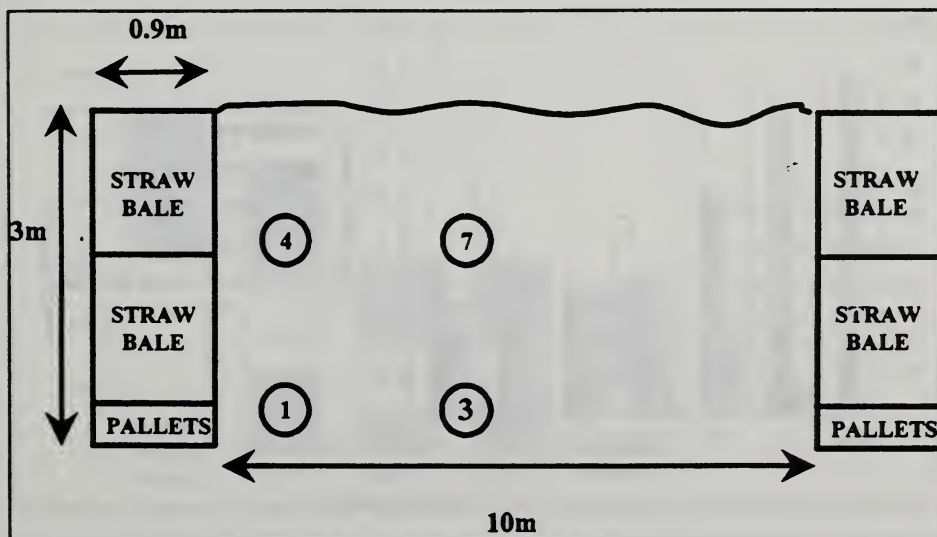


Figure 9. U.K. clamp cross-section, sample positions.

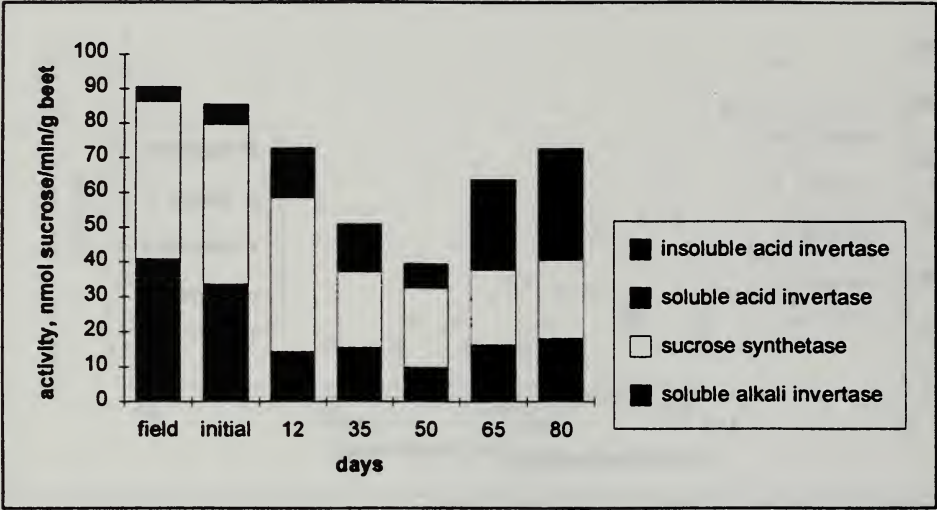


Figure 10. Individual invertase activities in sugarbeet during clamping.

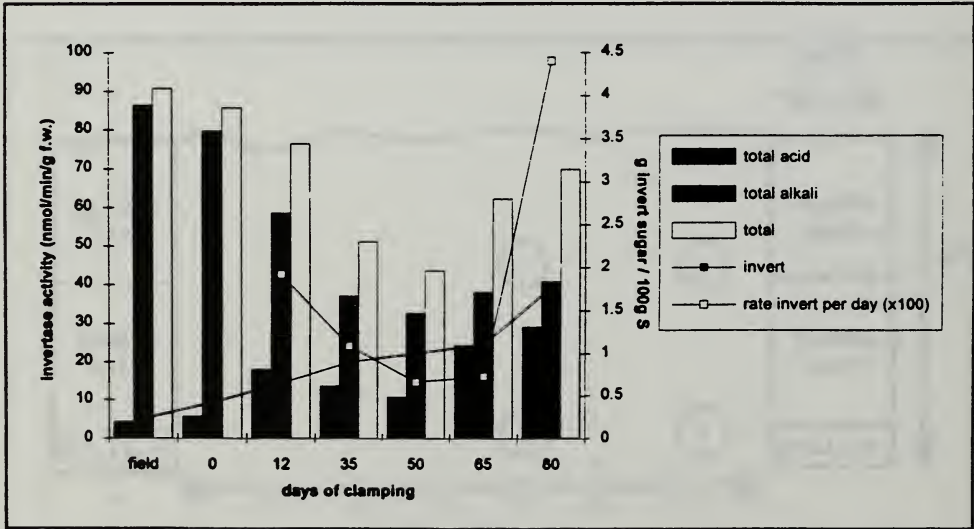


Figure 11. Invertase activities and invert sugar concentrations of sugarbeet during clamping trial.

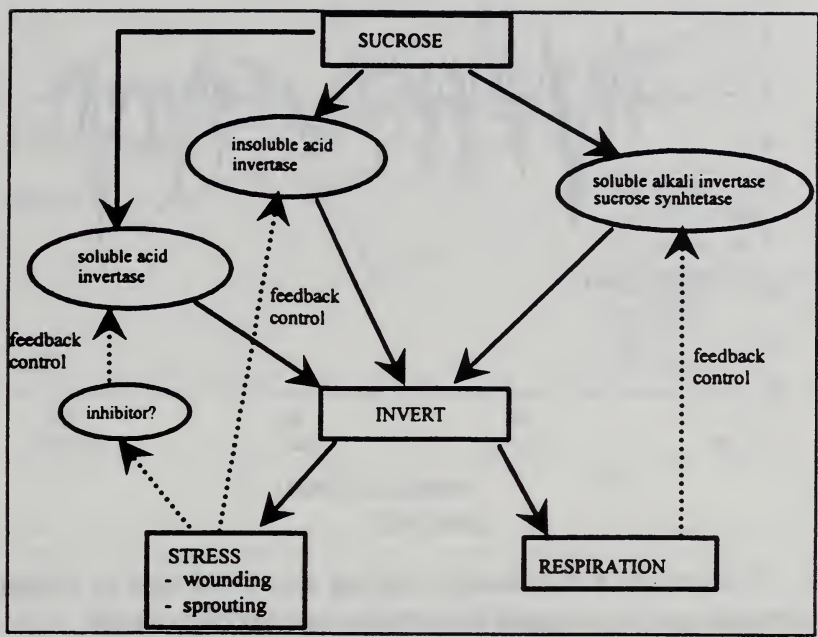


Figure 12. A model for the role of invertase and sucrose synthetase enzymes in sugarbeet during clamping.

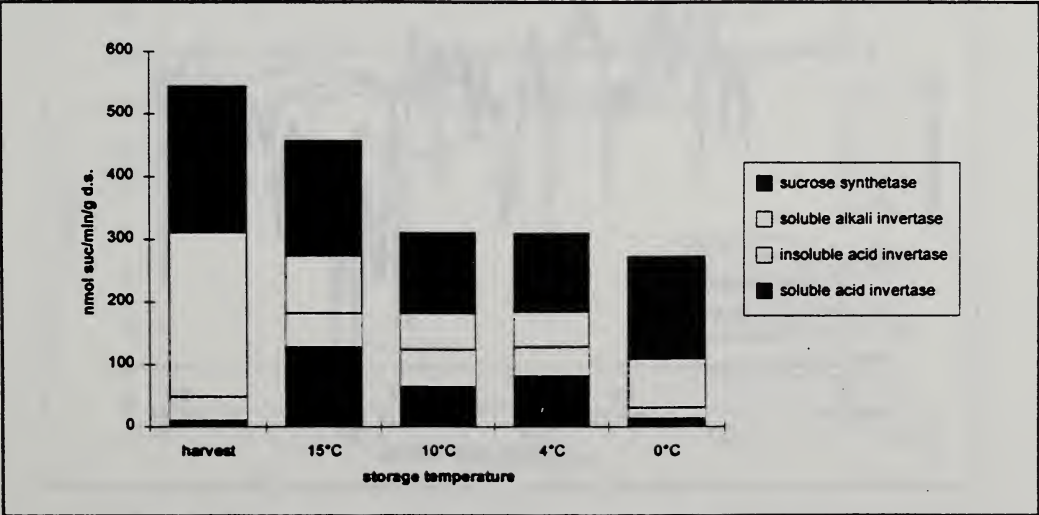


Figure 13. Invertase activities after 80 days storage at different temperatures.

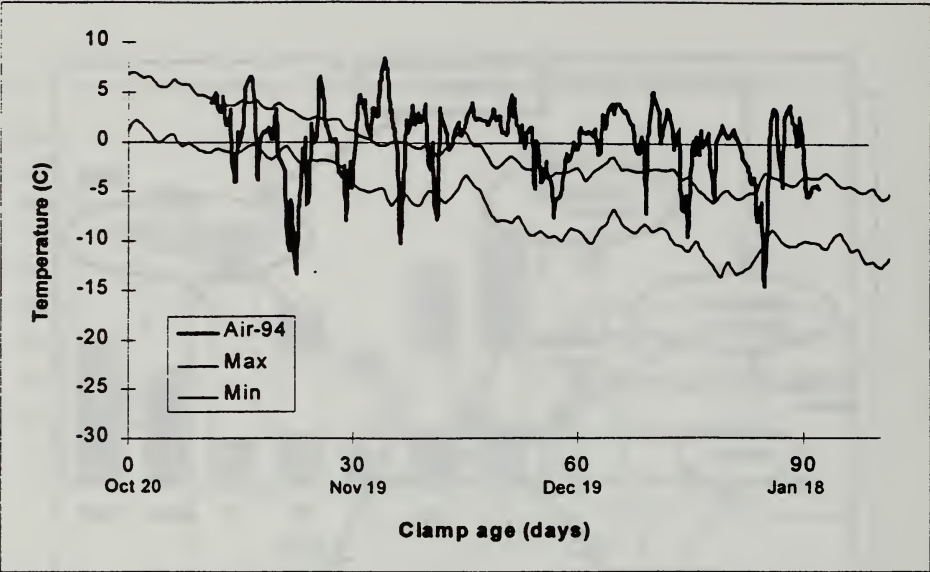


Figure 14A. Temperature fluctuation during the 1994 test in Finland. The daily average maximums and minimums were from years 1966 - 1996.

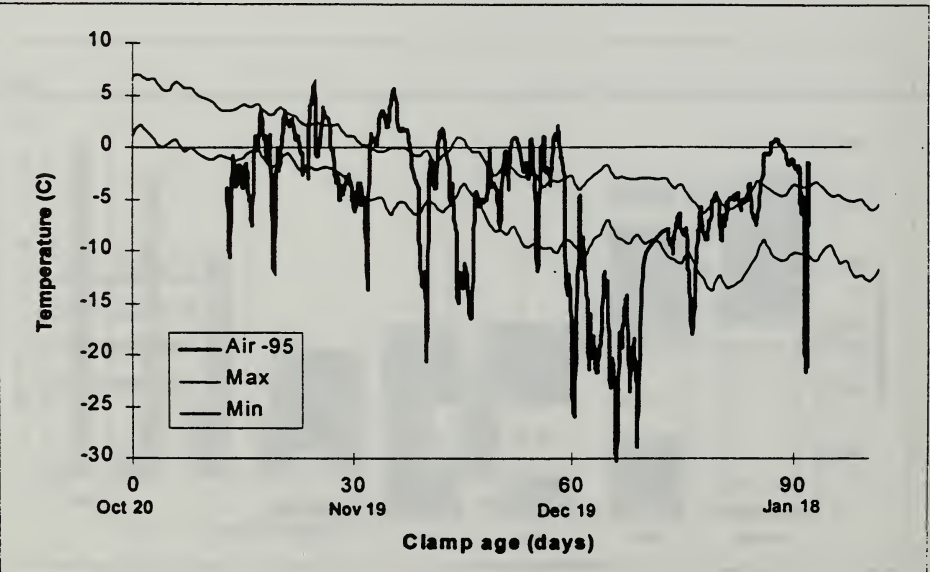


Figure 14B. Temperature fluctuation during the 1995 test in Finland. The daily average maximums and minimums were from years 1966 - 1996.

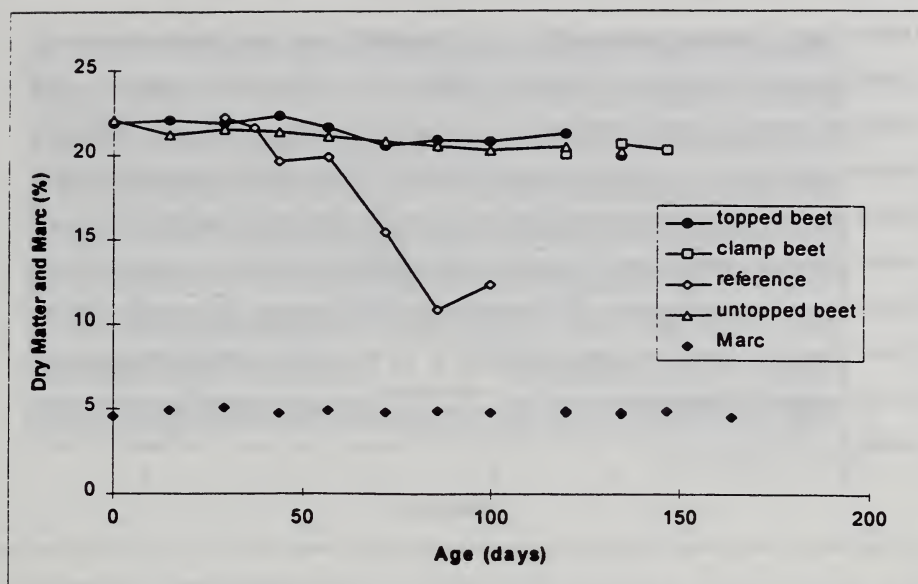


Figure 15. Total dry matter and marc during 1994 test in Finland. Marc values from different samples were close each others and are not separated.

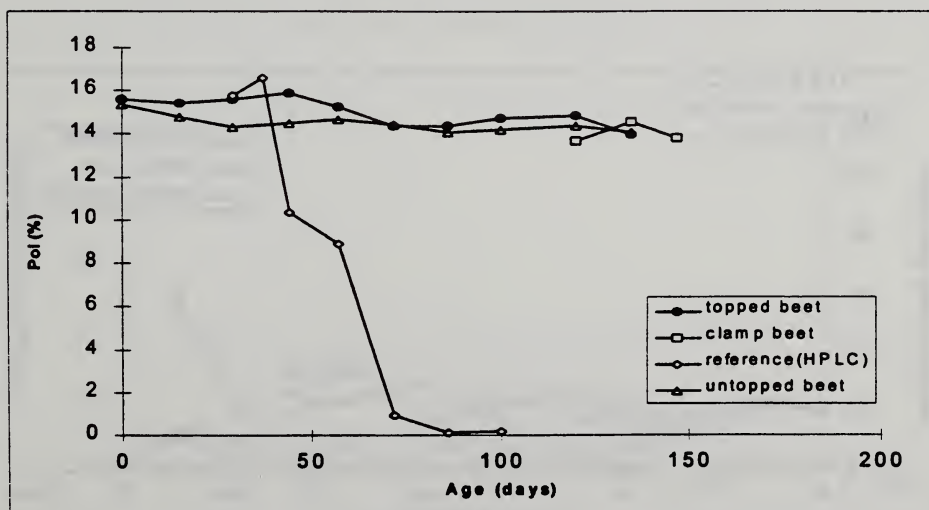


Figure 16. Pol of the beet samples. (Reference samples are assayed with HPLC due to the filtration problems in Pol assay.) (Finland 1994)

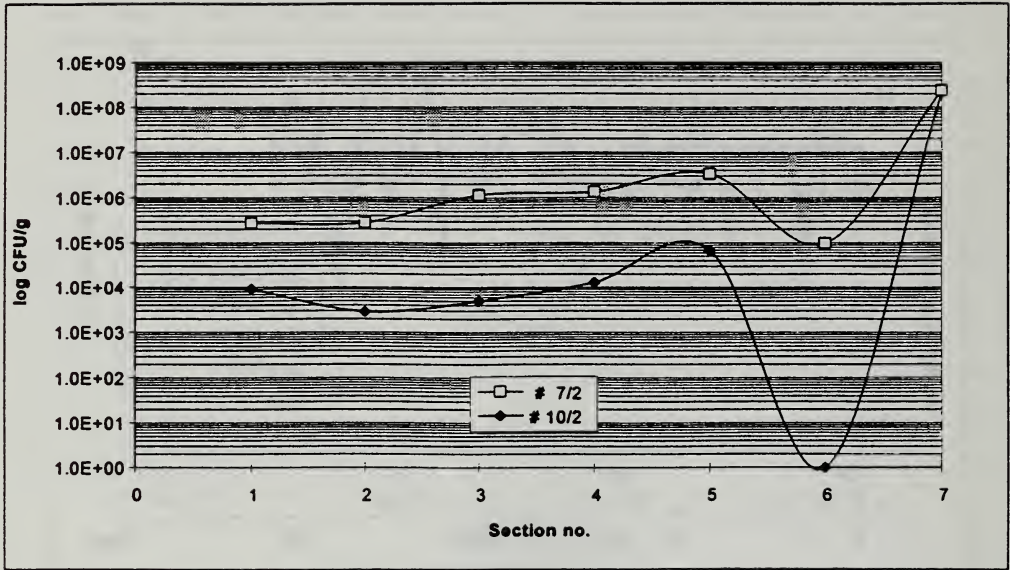


Figure 17. Distribution of microorganisms in two sectioned beet samples, sample #10/2 a good quality root and #7/2 in the stage of very early spoilage. Section 1 in the center of the root and section 7 the skin. (Finland December 1994) (Courtesy of Robert von Weissenberg, Cultor Technology Center, Kantvik)

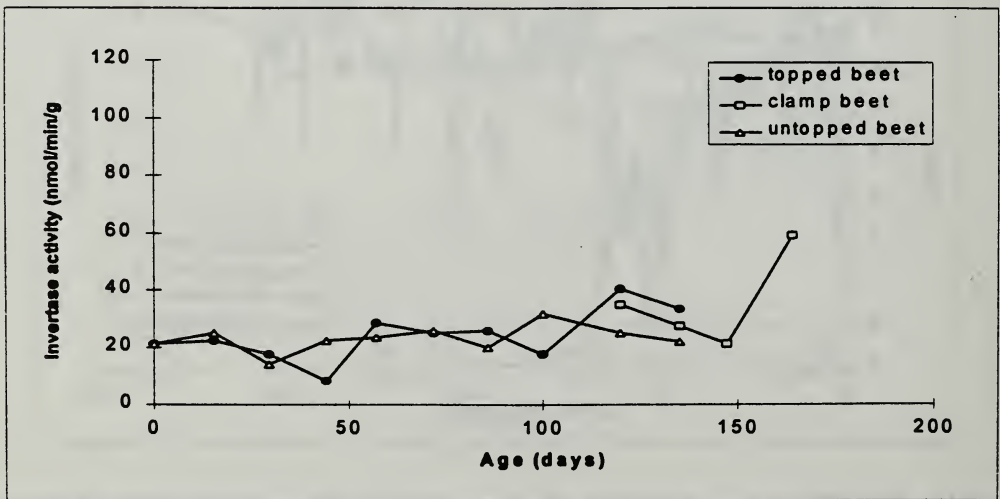


Figure 18. Alkaline invertase. (Finland 1994)

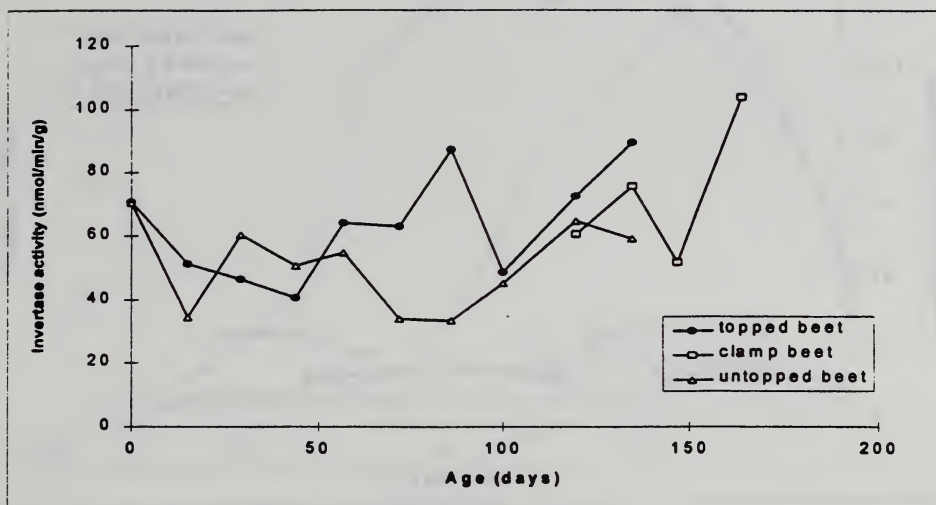


Figure 19. Sucrose synthetase. (Finland 1994)

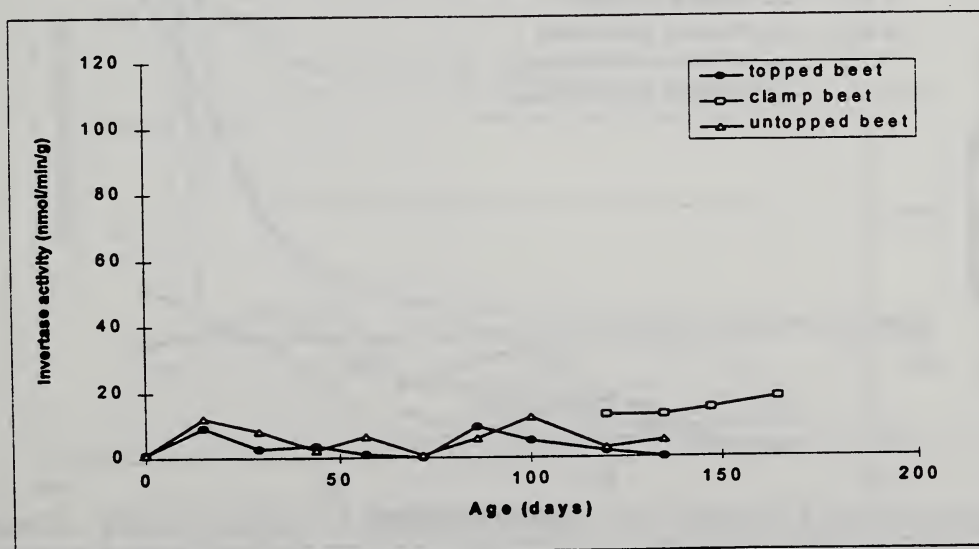


Figure 20. Soluble acid invertase. (Finland 1994)

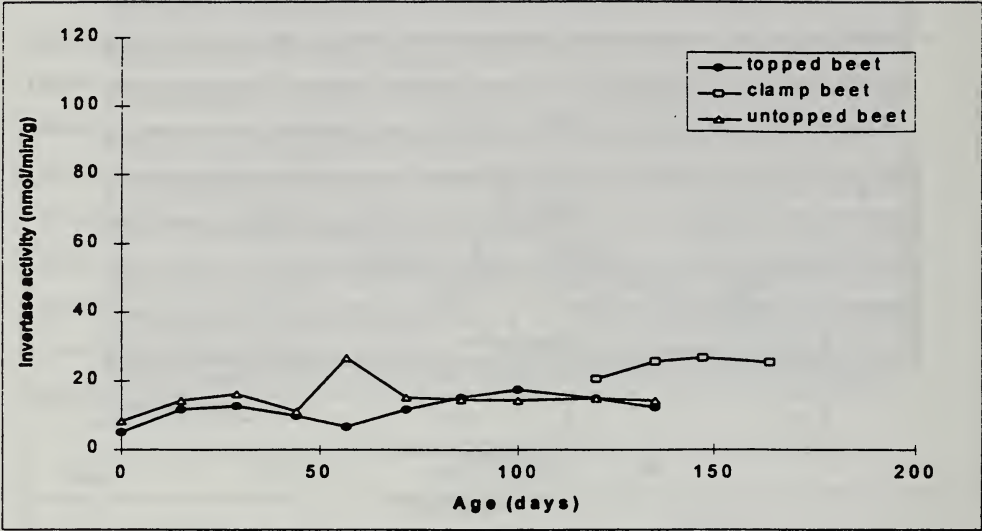


Figure 21. Insoluble acid invertase. (Finland 1994)

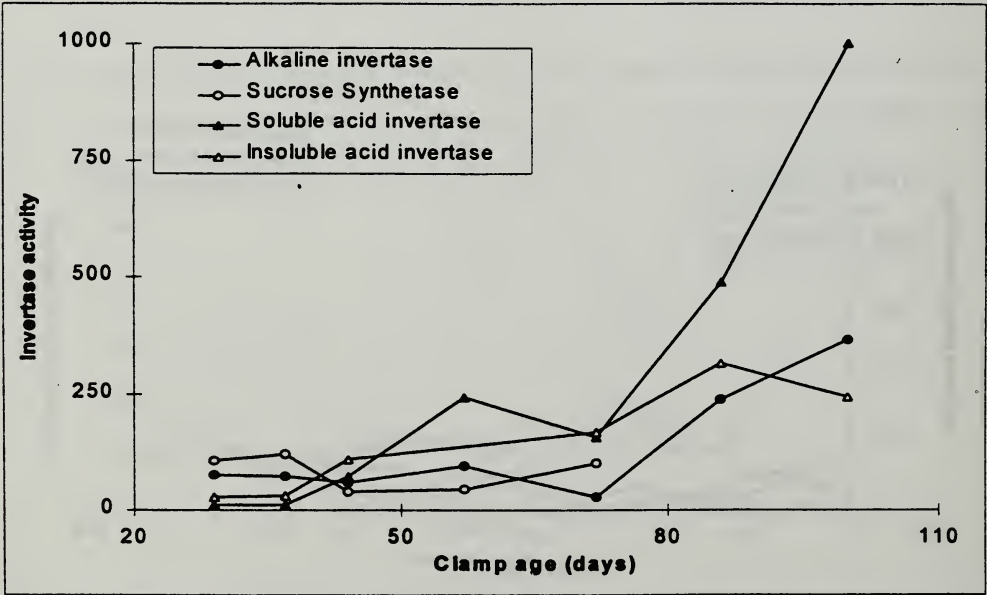


Figure 22. Invertase activities in spoiling reference samples. (Finland 1994)

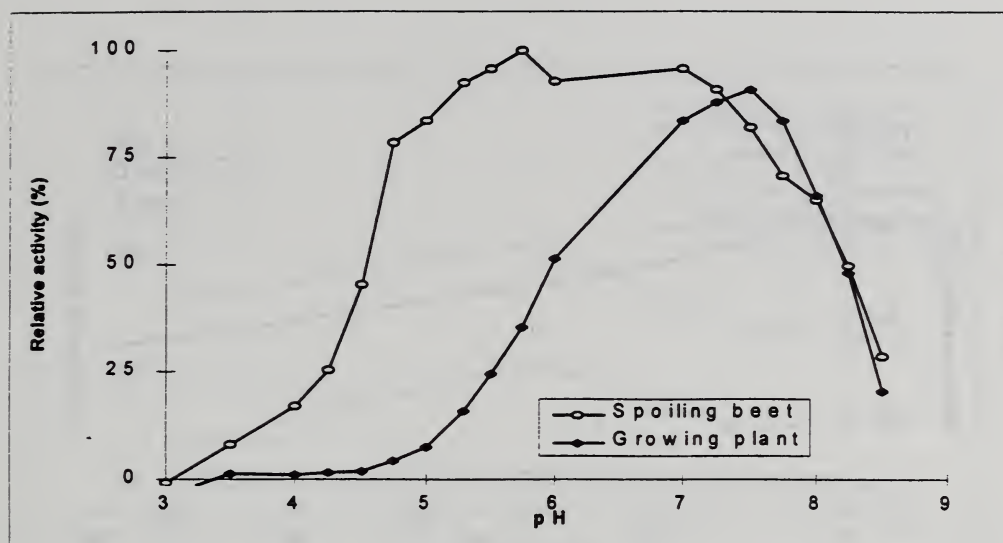


Figure 23. Relative total invertase activity expressed in a growing root and in a root during an early stage of spoiling at different pH values. (Finland 1993)

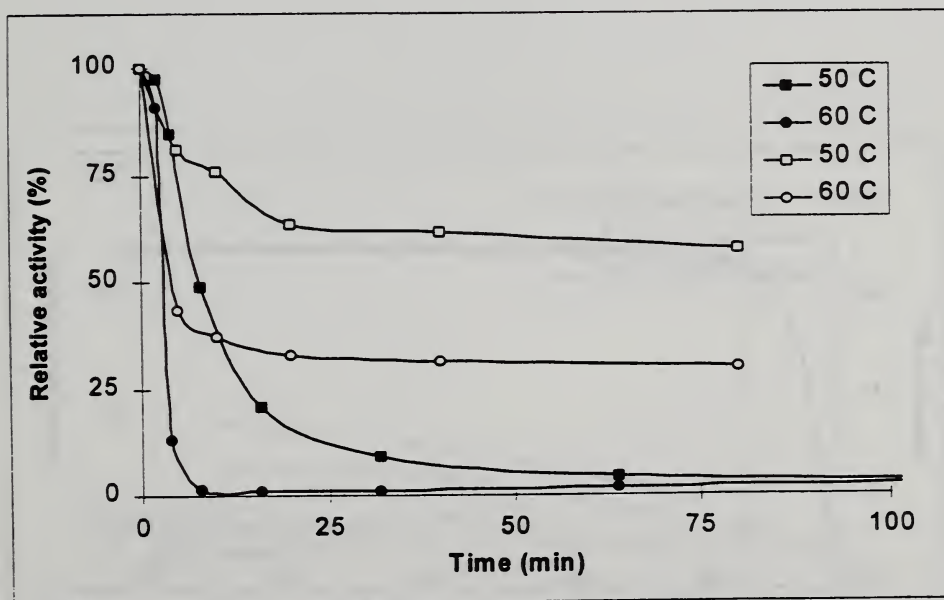


Figure 24. Thermostability of alkaline invertase of an unspoiled growing beet (solid marks at 50 and 60 C) and of a beet during an early stage of spoiling (open marks at 50 and 60 C) (Finland 1993).

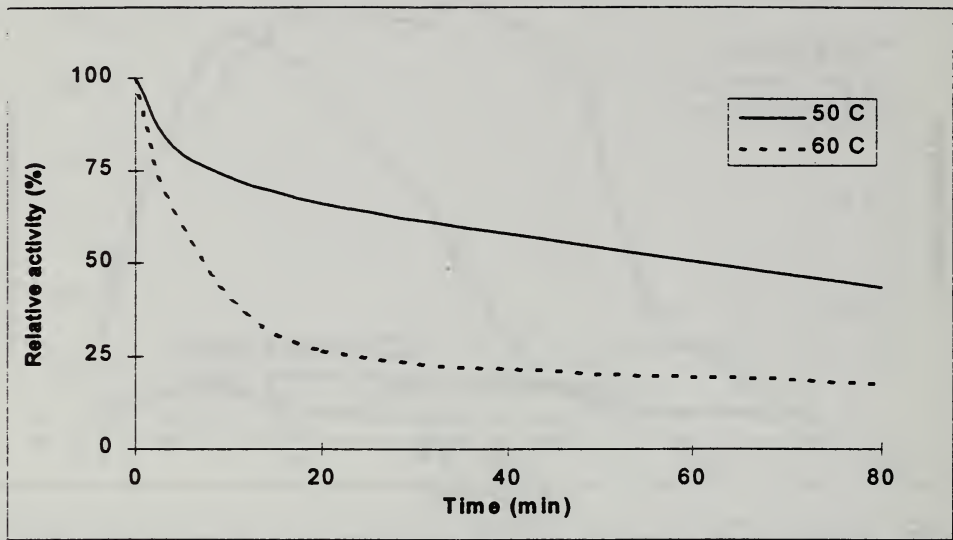


Figure 25. Thermostability of insoluble acid invertase of a beet during an early stage of spoiling. (Finland 1993)

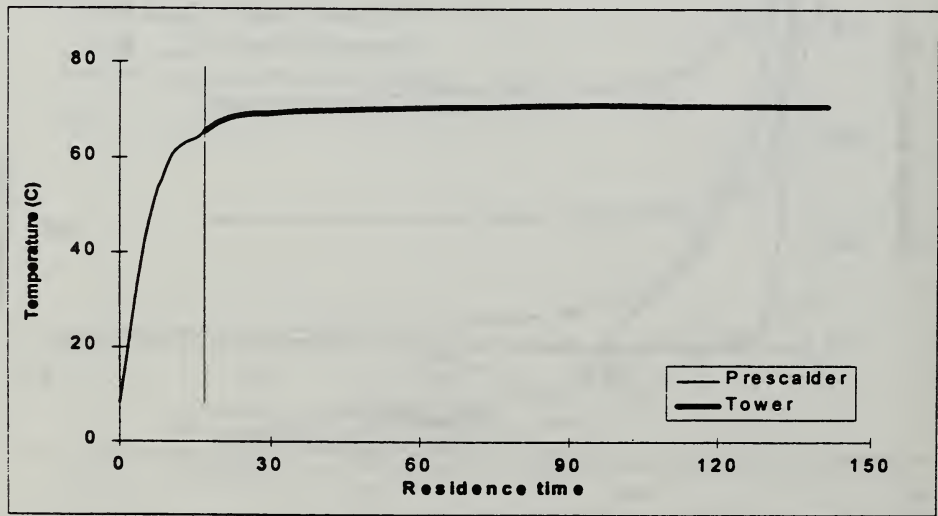


Figure 26. Temperature and residence time (min) of the diffusion unit in Salo factory. (Finland 1994)

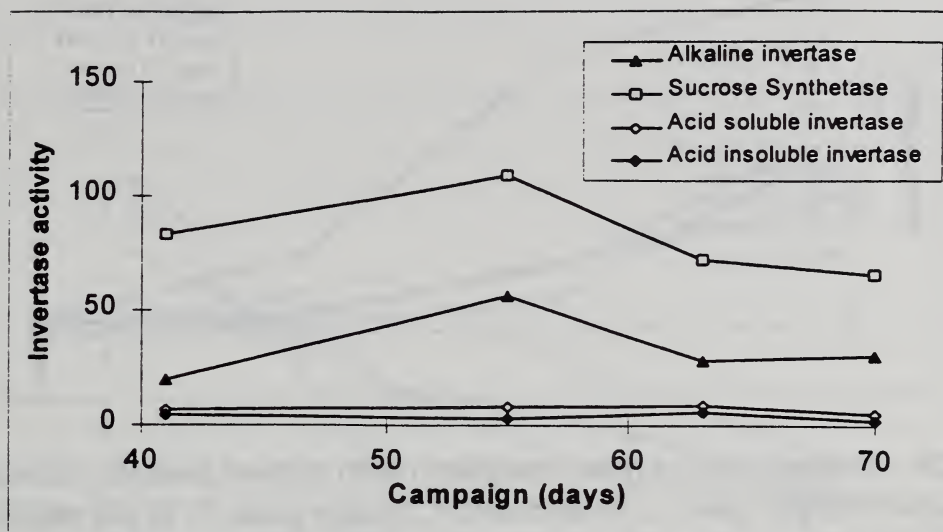


Figure 27. Invertase activities (nmol/min/g fresh beet) in fresh cossette fed to the prescalder. (Salo factory, Finland 1994)

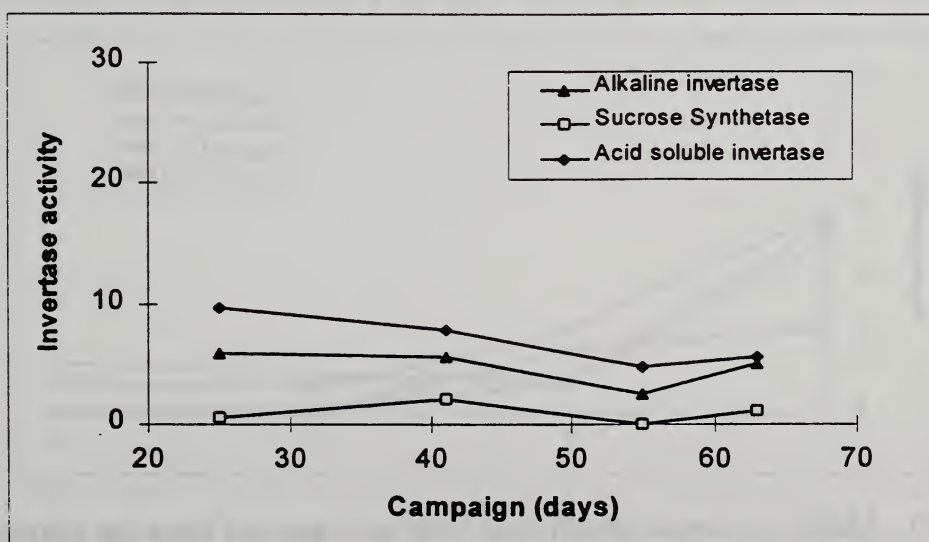


Figure 28. Invertase activities (nmol/min/g fresh beet) in raw juice drawn from the prescalder. (Salo factory, Finland 1994)

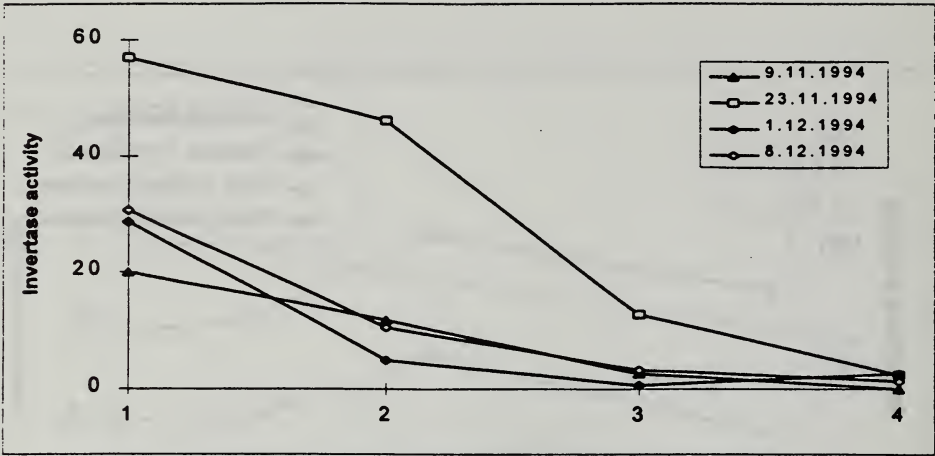


Figure 29. Alkaline invertase (nmol/min/g fresh beet) assayed from the cossette in the prescalder. Sample point 1: fresh cossette; Sample point 2: in the middle of the prescalder's cold part; Sample point 3: in the middle of the prescalder; Sample point 4: in the middle of the prescalder's hot part. (Salo factory, Finland 1994)

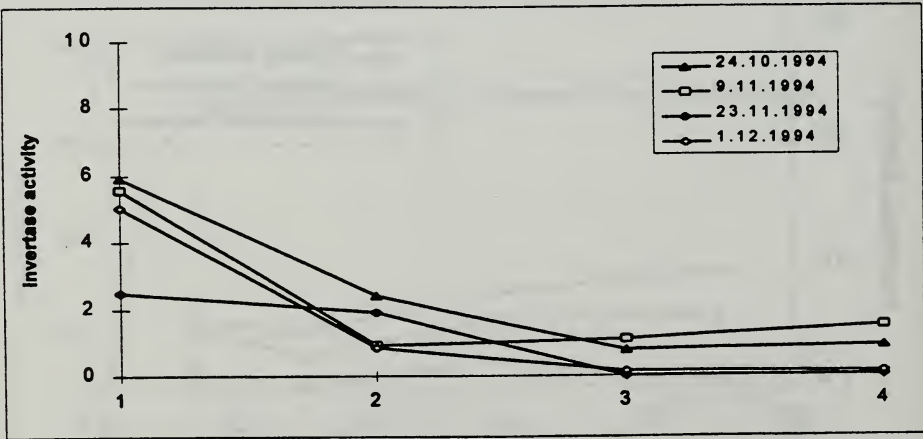


Figure 30. Alkaline invertase (nmol/min/g fresh beet) assayed from the solution in the prescalder. Sample point 1: raw juice; Sample point 2: in the middle of the p-prescalder's cold part; Sample point 3: in the middle of the prescalder; Sample point 4: in the middle of the prescalder's hot part. (Salo factory, Finland 1994)

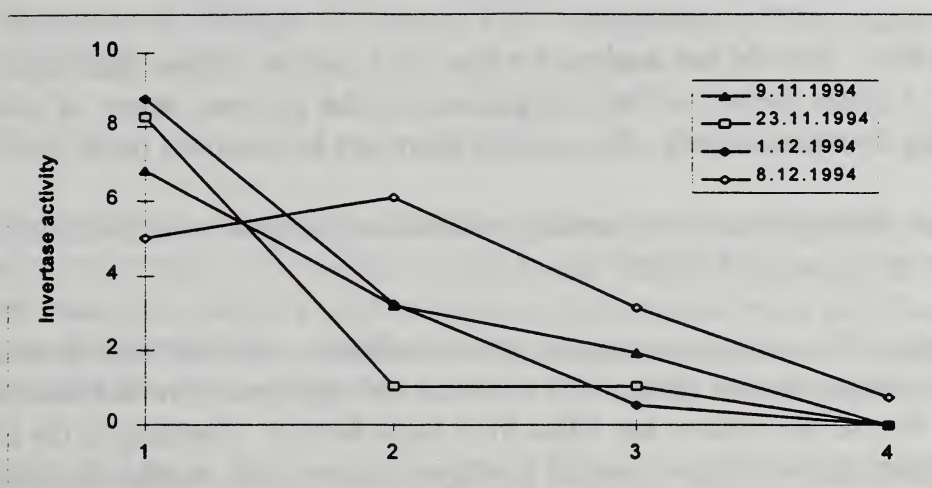


Figure 31. Acid soluble invertase (nmol/min/g fresh beet) assayed from the cossette in the prescalder. Sample point 1: fresh cossette; Sample point 2: in the middle of the prescalder's cold part; Sample point 3: in the middle of the prescalder; Sample point 4: in the middle of the prescalder's hot part. (Salo factory, Finland 1994)

DISCUSSION

Question: Did you consider any invertase activity coming in with the flume water that adheres to the beets?

Walliander: We did not analyze for that, so I cannot address that. But we did observe a great variety of microorganisms in the process, many of which are expressing invertase activity. So certainly there can be invertase there.

Question: Have you made any studies on the size of the roots and invertase activity, or on the soil type and activity?

Elsegood: No, we have not done size specifically. We did have three or four different varieties in the study, with different soil types, and we did measure root weights. We did not observe any effect from those factors. Certainly in the U.K. the seed breeders do not concentrate on invertase factors - they emphasize sodium and potassium. This work was directed to them.

Walliander: In the work in Finland, we did consider beet size, but were unable to establish any connection between beet size and invertase activity.

MOISTURE CONTENT OF SUGAR CRYSTALS AND INFLUENCE OF STORAGE CONDITIONS

Jan Maarten de Bruijn and Arno A.W. Marijnissen, CSM Suiker bv, The Netherlands

ABSTRACT

After the centrifugals, the drying and cooling process, sugar crystals still may hold an amount of water; up to 0.07 %. This so-called total water of sugar crystals comprises inherent water (i.e. moisture pockets in the crystal), bound water and free water. During conditioning of sugar, the bound water converts to free water which then may evaporate for the vast part from the crystals, so lowering their moisture content.

A method has been developed, using Karl Fisher analysis, which enables us to distinguish between these three types of water found in sugar crystals. The principle of the method will be outlined.

In this way we followed the course of moisture in sugar crystals during storage in silo and in different packaging materials.

The influence of storage conditions, e.g. temperature and relative humidity, on the storability of crystalline sugar has been investigated. In this context the role of packaging material has also been taken into account.

The outcome of this research project is very useful in order to be able to understand what causes caking and stickiness of crystalline sugar. Furthermore, this knowledge can be applied to help preventing these undesired phenomena.

INTRODUCTION

Literature survey:

Many studies have been devoted to the (in)stability of crystalline sugar during storage. The interest for the storability of sugar originates from the problems which frequently arise concerning the reliability of long-term storage, either in silos, bags or domestic packets. Such problems are agglomeration of crystals, stickiness, caking, lump formation and loss of free flowing ability of sugar. As a consequence, it may be difficult to meet customer requirements, when sugar is removed from storage.

Additionally, removing the sugar from the silo after storage of several months may be cumbersome, due to caking/stickiness of the sugar.

The most important parameter to be considered for good storage is water. On the one hand, the water content of crystalline sugar entering storage will depend on the previous process steps, i.e. sugar crystallization, removal of syrup and washing in centrifugals, drying and cooling. On the other hand, part of the remaining water should be removed by adequate conditioning during storage.

With respect to the research already carried out on the theme of water in connection to storability of sugar, particularly the reviews of Bunert and Bruhns (3) and Mikus and Budicek (6) excellently describe the state of the art. Also the work of Wilson et al. (11) is noteworthy. However, the literature appears to be slightly confusing as far as the different kinds of water present in sugar crystals is concerned.

Free moisture is generally recognized as the water at the surface of sugar crystals, which is in equilibrium with the surrounding air (3, 10, 11). This is normally taken to be the water which is detected by a loss on drying analysis (11), but according to Piper (8) this method determines the bound water and non-water volatiles as well.

Bound water is considered to be subsurface water that is present in a saturated sugar solution trapped by a thin layer of amorphous sugar (3, 10, 11). Amorphous sugar is abruptly formed during the drying of sugar crystals as result of a too quick water removal at the crystal surface, e.g. by applying too high temperature of the drying air or due to supplying an excess of (too) dry air. Under such conditions the sugar solution rapidly reaches a high concentration in which the molecular association for crystalline sugar cannot be obtained. Thus a glassy layer of amorphous sugar and a viscous concentrate solution is formed (5, 6). The surface layer is at the root of instability of sugar crystals during storage, due to hydration, crystallization and dehydration reactions. At this stage bound water becomes free water and therefore can be released from the crystal surface. This phenomenon is known as sugar stabilization, ripening, maturing, or conditioning of the sugar. The exothermic effect of crystallization in the surface layer is sufficient to get rid of the bound water that has been converted to free water, provided that ventilation is made with fresh and dry air. If not, then the liquid bridges between sugar particles may change into solid bridges, resulting in the cementing of the crystals. Such joints can be very tenuous giving the sugar a crusty texture, or can be severe giving lumps which are impossible to break (2, 11).

Up to now it has appeared to be difficult to distinguish analytically between free and bound water. Therefore, both forms are usually determined as the sum, called surface (3, 11) or external (1, 8) moisture respectively water.

The third form of water is that included in the crystals, called either inner (3, 6), inherent (10, 11) or internal (8) moisture respectively water. This water is thought to be present as droplets of mother syrup which become trapped within the crystal lattice and those enclosed in the interstices of conglomerates (3, 6, 10, 11). Improvement of the technique of sugar crystallization by mechanically forced circulation of the massecuite largely shifted the crystal shape from complicated conglomerates (as produced in ordinary vacuum pans) towards almost all crystals present as well-shaped single crystals, with singularly few conglomerates (10). Apart from a reduction of inherent moisture, Rodgers and Lewis (10) demonstrated that also the amount of moisture released during conditioning, i.e. free and bound water, is significantly lower for crystals obtained from forced circulation pans.

Research objectives:

The interest in the moisture content of sugar at CSM Suiker originates from studies on the colour development of sugar during storage in silos. It is well-understood that the temperature during storage plays a major role in colour formation. In addition, we concluded that the number and size of included mother syrup droplets also considerably affects sugar colour during storage. In order to be able to determine the inclusions, they first were visualized by heating the sugar for 45 hours at 105°C. Then, the number of the highly coloured inclusions could be determined by microscopic screening of the sugar sample.

As this approach is rather cumbersome and time-consuming, we developed a more convenient method, based on Karl Fischer analysis of water, which more or less confirmed the above-mentioned conclusion on colour formation during storage. The principle of our KF method will be outlined in this paper. It differs with respect to sample preparation from KF methods earlier reported in literature (1, 8). Furthermore, we have applied our new method for water analysis in sugar crystals for other purposes as well:

- In general to improve the basic knowledge on the presence of water in sugar crystals and its development during storage.

- Measurement of the water content of crystalline sugar in the process, with the aim to improve, i.e. to lower, the water content of freshly produced sugar before entering storage, if possible.
- Determination of the influence of storage conditions and packaging materials on the storability of sugar.

This paper summarizes the results of our research on the water content of sugar crystals and influence of storage conditions, carried out in the period 1994/95.

METHODS AND MATERIALS

Karl Fischer water analysis was performed with a Metrohm 652 KF coulometer and Hydranal-coulomat AD (Riedel-deHaën) as the reagent for coulometric KF titration. In order to be able to distinguish between the different forms of water in sugar crystals, sugar samples were treated in two different ways:

1. To 20 g sugar in a 30 ml glass bottle 15-20 ml methanol GR dried (Merck) was added. The bottle was closed by a screw cap which contained a rubber septum. The *free water* of the sugar was extracted by shaking the glass bottle manually for about 1 min. Then, the sugar crystal slurry was centrifuged for 5 min. at 900 rpm in order to obtain a clear methanol solution. From this solution a sample of approximately 2 g was drawn with a syringe, which was injected in the KF cell for water analysis.

The percentage of free water in the sugar sample was calculated from the analyzed amount of water and the determined weights of sugar, added methanol and injected methanol solution.

The water content of the dried methanol was determined as well, following the same procedure without sugar, and subtracted in the calculation as blank.

2. For analysis of the *total water* content of the sugar, i.e. the sum of free, bound and inherent water, it is necessary to grind the sugar crystals in order to release the *included* moisture comprising both bound and inherent water. For that purpose 15 stainless steel balls (6.3 mm diameter) were added to the glass bottle. After addition of the sugar and methanol, the sugar was milled for 30 min. using a

mechanical flask shaker (model SF1, Stuart Scientific) at maximum shaking frequency.

The particle size of the ground sugar was typically smaller than 40 μm .

The total water of the sugar, present in the methanol solution after this sample preparation, was determined as just described for the KF analysis of free water.

The included water, i.e. the sum of inherent and bound water, can be simply derived by calculating the difference from the analyzed total water and free water content.

The *bound water* content could be only obtained after conditioning of the sugar, which eventually gave information on the *inherent water* content present in the included moisture. This approach of bound water determination will be demonstrated below in more detail.

RESULTS AND DISCUSSION

Analysis of free water:

It must be noted that upon the methanol extraction of water from the sugar crystals also a small part of the sugar is dissolved. The sample preparation for the analysis of free water gives rise to a sugar concentration of 0.6 % (w/v), which is close to literature data for saturated methanol (7). Accordingly, approximately 0.6 % of the sugar will have been dissolved.

Using literature data for the surface area, weight and density of sugar crystals (9) it can be calculated that a layer of about 1 μm thickness at the surface of the crystal is dissolved in methanol. Dissolution of such a thin layer is not enough to reach the bound water in freshly produced sugar as it is covered by a thicker layer of amorphous sugar, which is estimated to be of the size of approximately 12 μm (6). In conclusion, our KF analysis of free water certainly will not include the bound water.

Conditioning of sugar in silo storage:

The course of conditioning of freshly produced sugar in silo storage has been determined. A typical example is given in Figure 1. It appears that within 2 weeks the sugar stabilizes in storage, which indicates that in the meantime the bound water has

been released from the crystals. Thus, after conditioning only inherent water (0.012 % in this example) in mother syrup droplets remains as the included water. The free water content rapidly decreases to 0.002 - 0.003 % and is in equilibrium with the surrounding air after 5 days of storage. The bound water that is released (as demonstrated in Figure 1) amounts to 0.017 % and the decrease of the free water content amounts to 0.009 %. In other words 0.026 % water on sugar, i.e. 260 ml water per ton sugar, has been vaporized and removed from the silo.

Influence of process conditions:

From the point of view that the more water removed in drying, the better the storability of freshly produced sugar, it may be interesting to look at the optimization possibilities of the drying process. Particularly the temperature (and humidity) of the drying air as well as the available respectively the utilized capacity of the drier may considered to be important parameters for adjustment of water removal. Therefore we studied the influence of both parameters, within the limits of the drier. The drier of concern was a rotary drier in counterflow operation, which consisted of two drums, one for drying and one for cooling.

First we investigated the influence of the temperature of the drying air. The effect of drying at a temperature around 86°C was compared to that at 59°C. The cooling section was constantly supplied with air of 16°C and 50 % R.H. Hourly samples were taken and analyzed at the laboratory for free and total water content. From their difference the included water, comprising both inherent and bound water, could be calculated.

In order to obtain data on the inherent water content, we dried all samples in an oven at 30°C for two weeks. Afterwards again the total and free water contents were determined. Their difference gave us the inherent water content only, as the bound water was released during conditioning in the oven. Knowing the inherent water content of the sugar samples, the bound water content could finally be determined by subtracting this inherent water from the included water content of the initial fresh samples. Also samples of the sugar entering the drier were taken and analyzed for the 'loss on drying' content according to the official ICUMSA method (4).

Figure 2 shows the results of this investigation. For the sake of clearness, only the relevant data are presented. As clearly demonstrated, neither the free water nor the bound water content of the sugar have been affected by the drying temperature.

Apparently, even at the lowest temperature (59°C) the layer of amorphous sugar is formed more or less at the same distance to the crystal surface as is the case at 86°C . In between the bound water has been trapped.

Furthermore, the amount of bound water appears to be independent of the original moisture content of the sugar, which fluctuated between 0.24 % and 0.53 % during the measurement. The inherent water content of the sugar (not shown) amounted to 0.014 % on an average, with 0.010 % and 0.021 % as the extreme values. The observed variation of the inherent water content will be due to the fact that each strike of batch sugar boiling (in different pans) may result in different amounts of included mother syrup in the crystals.

Secondly, the influence of the utilized capacity of the drier/cooler on the water removal was investigated. Starting with an empty drier, it was gradually filled with sugar coming from the centrifugals up to maximum capacity. Every 5 min. sugar samples were taken at the outlet of the cooling section. The free and total water contents, and from these the included water content, were determined. The course of these water contents is shown in Figure 3. Also the sugar temperature after cooling is presented, which will have been related to the filling grade of the drier/cooler. There seems to be a slight tendency to a lower total water content, as a result of a lower included water content, at a higher sugar loading of the drier/cooler. Possibly the water evaporation at low filling grades is quicker and may cause the inclusion of somewhat more bound water. This may be due to a large excess of drying air in comparison to the amount of sugar. However, the maximum difference in included water content at low and high sugar loadings of the drier is rather small (0.009 %) and seems to be hardly significant.

In conclusion, within the usual practical limits of the drier/cooler the final water contents of the sugar cannot be influenced to any extent.

Influence of packaging material on storability:

Knowing the hygroscopic property of crystalline sugar, not only an adequate conditioning in silo storage is required. Also the storage conditions in warehouses largely determine whether sugar in bags, sacks or small packages for household consumption will be susceptible to water vapour absorption or not. The upper safe limit for the storability of sugar is generally considered to be 60-65 % R.H. (3, 6, 11).

The packaging material may protect the sugar against a too humid environment. This ability to act as a barrier for water uptake by the sugar depends on the water vapour transmission rate of the packaging material. Usually synthetic coatings, like for instance HDPE, on paper are applied for this purpose.

As the waste disposal of used packaging materials has become a worldwide problem, nowadays there is a tendency to use repulpable paper products. As water vapour barrier a dispersion of recyclable and compostable modified polystyrene-butadiene copolymer can be applied as coating. We compared the applicability of a so-called monocoat material for packaging of sugar to that of commonly applied paper, coated with HDPE. We filled 25 kg sacks of both packaging types with an extra-fine granulated sugar (MA approximately 300 μm) and stored them in an airconditioned warehouse respectively in a non-conditioned environment. The water contents of the sugar were followed during two months; see Figure 4. After this period the differently stored sugars were checked on stickiness and lump formation.

In the warehouse provided with airconditioning no significant change in water contents was observed. In the non-conditioned storage, on the contrary, considerable water vapour absorption (and desorption) could be ascertained, which obviously was related to the variation of the relative humidity. The paper with HDPE coating protected the sugar quite well for a too rapid water absorption. The monocoat material, however, appeared to be hardly a barrier for water vapour as demonstrated by the considerable increase respectively decrease upon changing conditions. Consequently, the monocoat material contained after 2 months of storage a completely hardened sugar, with solid bridges between the crystals. No caking of sugar was observed in the HDPE coated packages as well as in the monocoat sacks stored in the airconditioned warehouse. From these results it can be concluded that either the monocoat material is unsuitable for packaging of sugar or the humidity during storage have to be maintained below 60 % R.H. Anyway, this method of testing the storability of sugar has demonstrated to be very helpful in coming to a decision concerning both the best choice of packaging material and the specification of storage conditions.

CONCLUSIONS

The method we developed for KF water analysis in crystalline sugar enabled us to differentiate between the different kinds of water present in the crystals, i.e. free, bound and inherent water.

We found values up to 0.07 % of total water in freshly produced sugar. As an example, within two weeks of silo storage the sugar stabilized by removal of 0.026 % water.

It appeared that at usual operation conditions of the factory drier/cooler it is not possible to influence the final water content of the sugar. It may be interesting to investigate the drying of sugar more thoroughly at pilot scale, in order to be able to vary parameters like temperature, air flow and humidity over a wider range, without the need to worry about the sugar quality.

The comparison of the storability of sugar in 25 kg sacks at different environmental conditions confirmed that only at a relative humidity below 60 % the water content of sugar remains unchanged. If sugar is exposed to higher relative humidities, a suitable coating on the packaging material will protect the sugar against a too rapid absorption of water vapour. The developed method for KF water analysis can usefully be applied for determination of a proper choice of the packaging material that is required.

ACKNOWLEDGEMENTS

The authors wish to thank Professor Mohamed Mathlouthi (University of Reims), who acquainted us with the fundamentals of the physical-chemical properties of sucrose and for helpful discussions.

Mr. Frans Caljouw (CSM Suiker) is acknowledged for performing the preliminary study on colour formation in silo storage; he laid the foundation of the new KF method of water analysis in crystalline sugar.

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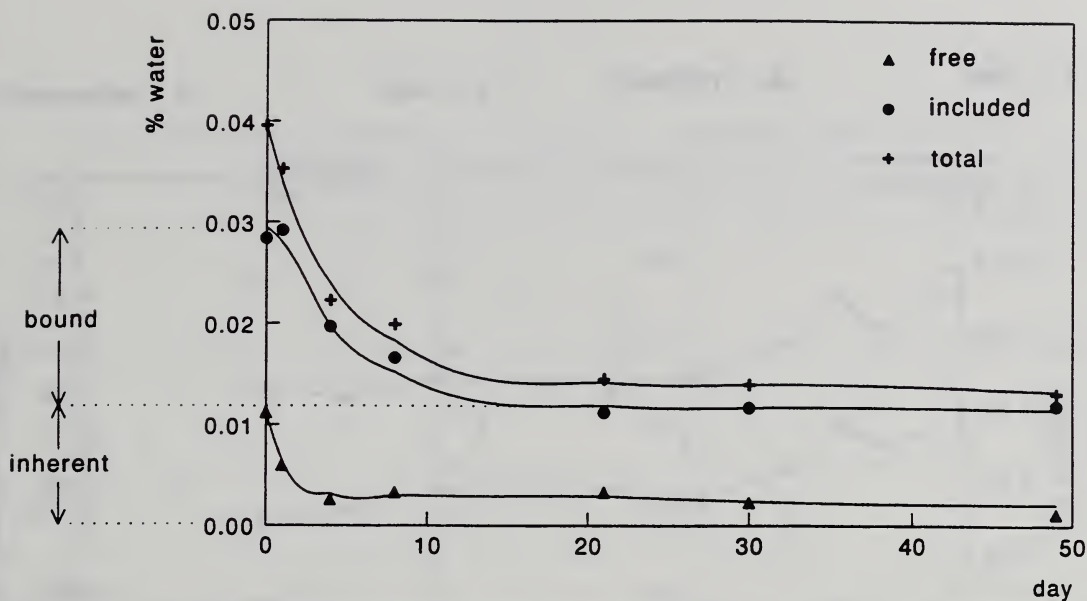


Figure 1. Conditioning of crystalline sugar in silo storage.

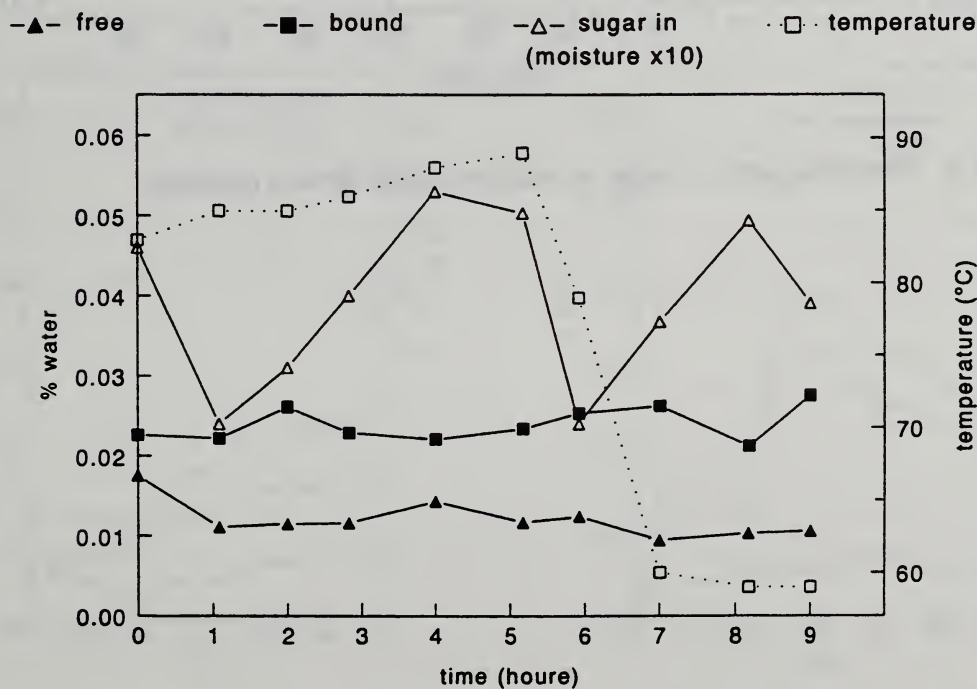


Figure 2. Influence of the drying temperature on the water content of sugar (sugar in = sugar from the centrifugals entering the drier).

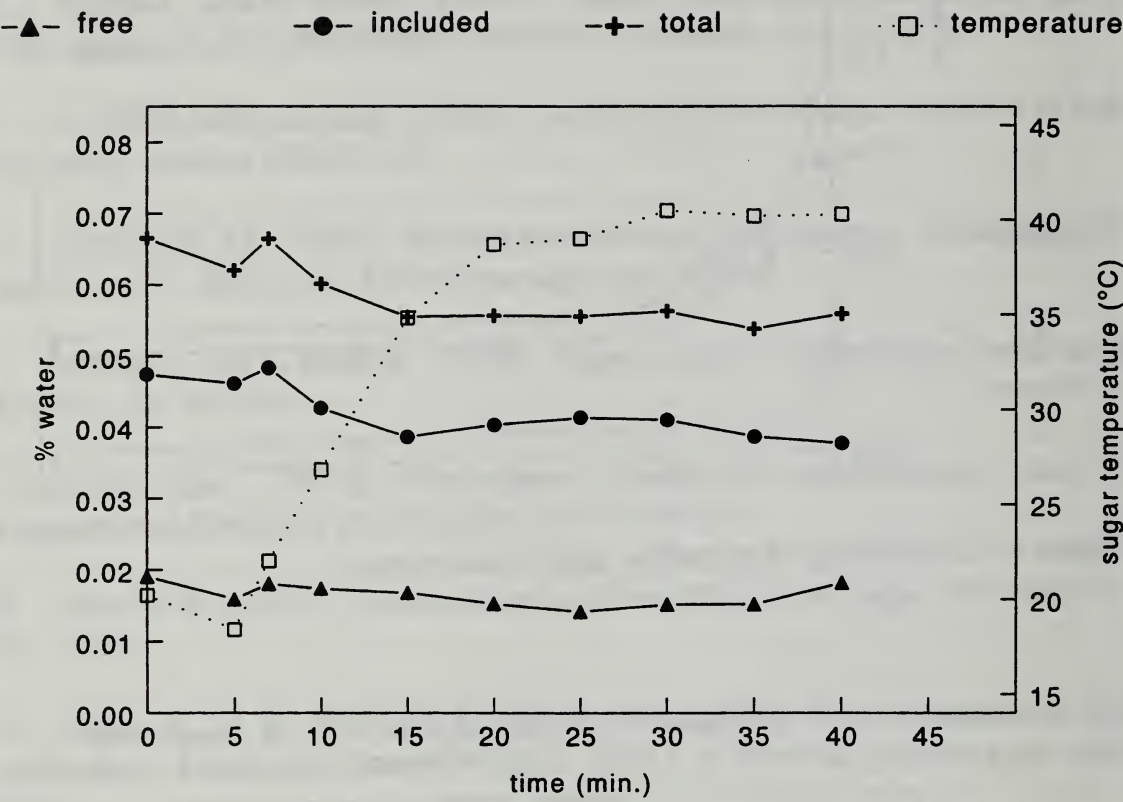


Figure 3. Water content of sugar in relation to the drying capacity.

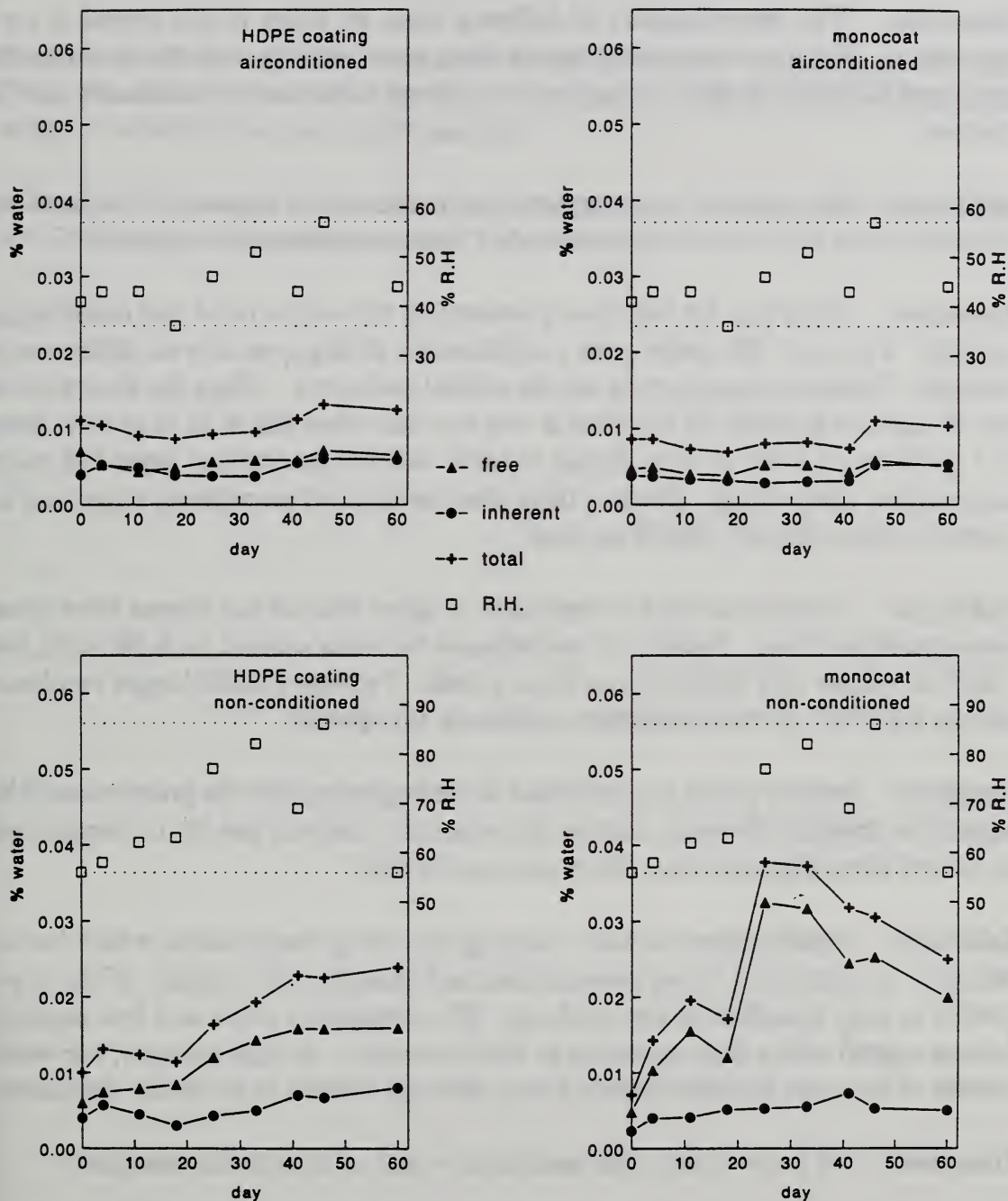


Figure 4. Influence of packaging material on the water content of sugar at different relative humidities.

DISCUSSION

Question: This determination of different kinds of water in the crystal is very interesting. Have you compared results from your method with results from the proposed ICUMSA method, using two subsequent titrations, at 11 minutes and 22 minutes.

deBruijn: No, we have not compared our method with that one. Nor have we compared ones with oven-drying methods. These comparisons are planned.

Question: Thank you for this clear presentation of water around and inside sugar crystals. You said that under your conditions for drying, you saw no difference in humidity. Perhaps temperature is not the critical parameter. When the dryers are in use, the relative humidity (R.H.) of air is very low, and when this R.H. is so low, there is a gradient of R.H. so that drying is rapid and the amorphous layer has more importance, after drying. Do you think that the layer of amorphous sugar can be controlled after drying? And if so, how?

deBruijn: Probably it can be controlled. I agree that all our dryers have some overcapacity in drying. Possibly we can influence the water content on a lab scale, but I think it will be very difficult on a factory scale. Perhaps a much longer residence time in the dryer, at more moderate conditions, is required.

Question: Another point: you indicated in the beginning that the process could be adjusted to minimize the water content discrepancies - that you can act on the process to control these discrepancies. How can you do this?

deBruijn: Modifications include changing the drying temperature, which has no influence as mentioned in my presentation, and changing the capacity of the dryer (which is only described in the handout). We compared a dryer at a low capacity (almost empty) with a dryer operating at 100% capacity. At high capacity, the water content of the sugar becomes slightly lower, although it seems to be hardly significant.

Question: Did you see any other parameters - size of crystal, for example?

deBruijn: No, not yet.

SPRI

Question: You said that you reached stability after two weeks of silo storage. Could you describe the conditions of your silo storage - whether you recirculate the sugar, or whether you use conditioned air?

deBruijn: The sugar is not recirculated. For aeration, the outside air is heated to 30°-35°C, which then corresponds to 30° - 40° R.H.

ODOR IN BEET SUGAR: SOME CAUSATIVE AGENTS AND PREVENTATIVE MEASURES

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ABSTRACT

The characteristic odor of beet sugar was examined using several different approaches. The objectives of this study were to identify specific sugar odorants, determine ways to prevent odorant formation, and develop processes to remove odorants from beet sugar. Odorants were isolated from test samples using liquid/liquid extraction and solid-phase adsorbents (e.g., Empore Disks, Optipore Resin), then characterized by GC, IC, MS, and sensory analysis. Over 40 odorants were identified in sugar, syrups, and wash waters. These included volatile fatty acids, hexanal, vanillin, α -terpineol, several pyrazines, and many other compounds. Centrifugal wash water and evaporator condensates were found to contain geosmin and MIB, compounds which have earthy, musty odors which may be imparted to beet sugar.

Factory air samples were found to contain odorants that may impact sugar quality. Longitudinal studies demonstrated that sugar odorants dissipate into the atmosphere over time, re-enforcing the importance of proper sugar conditioning. Inorganic adsorbents (e.g., zeolite) were shown to remove odors from beet sugar. The potential for these and other ameliorative measures is discussed.

INTRODUCTION

Beet sugar is known to have a characteristic odor. The presence of the odor is an occasional occurrence and can have a negative impact on beet sugar quality. The problem is especially noticeable with sugar that has been stored for long periods in leak-tight containers in which vaporization of odorants is prevented. The consequences of the odor are product dissatisfaction and consumer complaints, and can result in lost sales due to customers switching to alternative sweeteners.

Many causes of beet sugar odor have been suggested. These include (Table 1) such things as poor beet quality, microbial infections, odorants in wash water and factory air, beet deterioration during storage, and other causative factors.

The odor of beet sugar has been described as grassy, "green," barn-like, rancid, sour, earthy and musty, and is likely due to combinations of volatiles emanating from the crystals. In recent years, several laboratories have attempted to isolate and identify these compounds. Substances identified to date include organic acids, alcohols, aldehydes, pyrazines, and geosmin (7,8,15). Organic acids, produced during microbial infections, have a sour, rancid aroma, while aldehydes, formed by enzymatic oxidation of fatty acids, produce green/grassy fragrances (8). Pyrazines are nitrogenous compounds derived from amino acids and can have nutty, roasted, and earthy odors (10,11,14). Geosmin, a secondary metabolite produced by some microorganisms, is known for its earthy, musty aroma (5,6,14).

Recently, American Crystal has re-examined the problem of odor in sugar. Several different experimental approaches were taken with the ultimate objectives being to determine the identities and origins of those substances responsible for occasional customer complaints and to find ways to eliminate sugar odorants so as to improve our product.

MATERIALS AND METHODS

Procedures for odorant isolation included the purge and trap method, liquid-liquid extraction, and extraction with solid phase adsorbents. Analysis was by gas chromatography, ion chromatography, and sensory evaluation. In addition, the analytical services of several outside laboratories were also used. These included the Univ. of Minnesota Food Science Dept. in St. Paul, MN; Montgomery-Watson Labs in Pasadena, CA; and the Philadelphia Suburban Water Company in Bryn Mawr, PA.

Gas Chromatography

A Hewlett-Packard HP5890 Series II gas chromatograph (GC) was used for evaluation and identification of sugar odorants. The following columns were used: (a) Supelco SPB-1, 30 m by 0.32 mm ID; film thickness 1.0 μm ; (b) Alltech DB-5 Megabore column, 30 mm x 0.53 mm ID; film thickness 1.5 μm . Injector and detector temperatures were 200° and 250°C, respectively. The initial column temperature was 35°C, ramped to 220°C at 3°C/min. Injections were made using an

HP7673 Auto Injector. Volatiles were detected by means of a flame ionization detector (FID). Helium was used as the carrier gas.

Ion Chromatography

Sugar volatiles were detected on a Dionex DX-300 Ion Chromatograph equipped with a Dionex AS-11 column. Test samples were diluted with cartridge-purified deionized water prior to analysis. Analytes were resolved by gradient elution @ 2 ml/min using water and NaOH (5 and 100 mM) in ratios specified by Dionex. Detection was by conductivity.

Purge and Trap

One hundred grams of sugar crystals were packed into a jacketed glass condenser, then sparged with pure, dry nitrogen for 80 min. During sparging, the sugar was "fluffed" to prevent channeling by turning the condenser end for end once every 5 min. Water @ 65°C was circulated through the water jacket to heat the sugar. Odorants released in this step were trapped on a Tenax-TA trap. Sugar volatiles were then desorbed (@ 250°C) from the Tenax into an HP5890 GC by means of an Envirochem Model 850 Thermal Tube Desorber. Chromatographic separation was performed as described above, using the DB-5 Megabore column. The eluent stream from the GC was split, sending half of the volatiles to an FID to obtain a separation profile. The rest were diverted to a sniffer port for olfactory evaluation by a human subject to produce a sensory profile, or aromagram.

Longitudinal Studies

The effects of conditioning on sugar quality were tested by exposing malodorous sugar to clean air at ambient temperature for 32 days. At periodic intervals, sugar odorants were isolated by the purge and trap method. The levels of volatiles as a function of conditioning time were compared by GC.

Chemicals

Pyrazine and organic acid standards were obtained from the Sigma Chemical Co., St. Louis, MO; and from Aldrich, Milwaukee, WI. Geosmin and 2-methylisoborneol (MIB) were purchased from Wako Chemicals USA, Inc., Richmond, VA. All other chemicals used in this study were reagent grade.

Solid Phase Adsorbents

Empore Extraction Disks (Mfd. by the 3M Company, St. Paul, MN) were obtained from J. T. Baker, Inc., Phillipsburg, NJ. Three types of Empore Disks were used: (a) Empore Anion Exchange-SR (AE-SR); (b) Empore Octadecyl (C_{18}); (c) Empore Polystyrene-divinylbenzene (SDB-XC). Optipore resin was obtained from the Dow Chemical Company, Midland, MI. Prior to use, adsorbents were conditioned according to the manufacturer's recommendations.

ABSCENTS^R deodorizing powders, beads, and tiles were obtained from UOP Molecular Sieve Division, Des Plaines, IL. All three forms of adsorbent were tested. Deodorization of sugar by ABSCENTS was performed by placing cloth packets containing 1 g of the adsorbent into glass jars with 50 g of sugar (Alternatively, the adsorbent was mixed directly with the sugar.). The jars were tightly capped and kept at room temperature. After ~1 hour, the jars were opened and the sugar evaluated by sensory panel.

RESULTS AND DISCUSSION

Purge and Trap

Purge and trap experiments were performed to correlate identities of sugar volatiles with specific recognizable odors. Figure 1 shows an aromagram obtained from white sugar using the purge and trap method. The peaks represent individual compounds resolved by gas chromatography and serve to illustrate that white sugar contains many volatile substances. These represent only a very small percent of the total material; however, they are present, and some have fragrances recognizable by the human nose, e.g., lemon, lake water, parmesan cheese, etc. Similar observations have been reported by other investigators (8).

The purge and trap technique was used to evaluate sugar conditioned by exposure to clean air over a period of several weeks. In GC profiles (not shown) obtained from sugar treated in this way, it was observed that odorant peaks grew smaller with conditioning time, indicating that sugar quality is influenced by proper conditioning and reinforcing the importance of this step for odor removal during sugar production.

The reduction in sugar odorants as a function of conditioning time was a predictable and expected result. However, it indicated that it might be possible to actively

promote odorant removal through the use of adsorbents. The UOP Corporation of Des Plaines, IL, manufactures a product called Abscents (Table 2). This is a molecular sieve made of zeolite and is sold in the form of powder, beads, and tiles. It is capable of adsorbing odorants from air or liquids and, after saturation with volatiles, it can be regenerated with heat.

Abscents was tested for its effectiveness in reducing sugar odor. It was observed that in <1 hour, Abscents removed all the odor from the headspace of sealed jars containing malodorous beet sugar. Although no quantitative assessment of this phenomenon was made, the results were encouraging and suggest that some additional evaluations of this material could be made. Abscents is a food grade product, and if found effective with sugar, it might provide an additional tool to deal with sugar odor. The cost effectiveness of using this product has not been explored.

Solid Phase Adsorbents

Concentration methods with solid phase adsorbents were also used in this study. Many chemicals responsible for food odors have very low odor thresholds, i.e., they can produce noticeable odors even when present in amounts that defy detection by sensitive analytical instruments. This makes it necessary to process large samples to recover measurable quantities of odorants. Solvent extraction methods are effective; however, they are labor intensive, time consuming, and can create waste disposal problems.

For this reason, beet sugar odorants were concentrated using solid phase adsorbents. These function like "sponges" to trap dissolved chemicals in aqueous solutions, and so they are suitable for processing large volumes of sample. One adsorbent used in this study was Optipore, manufactured by the Dow Chemical Company. This is a styrene-divinylbenzene resin derivatized with various functional groups and is used for decolorizing high fructose corn syrup. However, it also removes undesirable flavors and aromas. Small scale tests indicated that it could trap sugar odorants.

The procedure for processing test samples with Optipore is outlined in Figure 2. Optipore was suspended in distilled water, then poured into a glass column. A solution of the test material was passed through the resin to trap odorants. Unretained material was discarded; retained material was desorbed by washing the resin with methanol, then analyzed by GC.

One of the samples tested was white centrifugal wash water (WCWW). Prior to treatment, the water had a nutty, boiled corn odor and contained UV-absorbing material. The WCWW (39 L) was adjusted to pH 4.6, then passed through a jacketed column of Optipore resin (bed volume: 228 ml) at 60°C and a flow rate of ~1.2 L/hr. After treatment, the water had a sweet, corn syrup-like odor and negligible UV absorbance. After the entire sample had been eluted, the resin was washed at ambient temperature, first with ~3.5 L of distilled water, then with methanol (1.2 L), whereupon a yellow-colored fraction with a strong burnt odor was recovered. The methanol eluent was analyzed by GC.

The top panel of Figure 3 shows part of a GC profile of an Optipore extract of WCWW. As can be seen, the water used for washing white sugar contains many organic compounds. Some of the observed peaks had retention times corresponding to those of several known pyrazines. For confirmation purposes, the sample was spiked with pyrazine standards and re-run on the GC. The upper panel shows the GC profile of the wash water extract with presumptive pyrazine peaks indicated by the arrows. In the lower panel, it can be seen that when the extract was spiked with known pyrazines, these peaks became larger. The data indicate that the wash water contains 2-methyl-, 2,5- and/or 2,6-dimethyl-, 2,3-dimethyl-, 2,3,5-trimethyl-, and 2,3,5,6-tetramethylpyrazine. Pyrazines are formed by the reaction of glucose with amino acids such as glutamine and lysine, and are known to have nutty, roasted, and sometimes musty odors (10,11,14). Several of these compounds have been identified in beet factory process streams by other investigators (7,8,15,17). As such, our results are consistent with data previously reported in the literature.

Odorant adsorption was also accomplished using Empore extraction disks. These are manufactured by the 3M Company and consist of Teflon membranes derivatized with functional groups that bind specific organic compounds. The procedure for processing samples with Empore disks is shown in Figure 4. The test material was vacuum-filtered through the Empore disk and the filtrate discarded. Volatiles adsorbed by the membrane were recovered by solvent extraction, concentrated to small volumes, then analyzed by gas chromatography.

Empore disks were used to recover volatiles from white sugar. A sucrose solution (20% w/v, 2.5 L) was adjusted to pH ~2 with HCl, then filtered (as above) through an Empore SDVB membrane. Retained material was recovered by eluting the disk with 1 x 10 ml of acetone, then with 3 x 10 ml of CH₂Cl₂. The combined eluants were

dried with anhydrous Na_2SO_4 , concentrated under a stream of N_2 , then analyzed by GC.

Figure 5 shows part of a GC profile of the Empore extract of white sugar (lower panel). Of the many compounds in this sample, 3 had retention times corresponding to several of the pyrazine standards shown in the upper panel (namely 2,5- and 2,6-dimethyl-, 2,3,5-trimethyl-, and 2,3,5,6-tetramethylpyrazine), indicating that these substances are present in white sugar.

The data obtained from the use of Optipore resin demonstrated the presence of several pyrazines in WCWW (Fig. 4). With Empore disks, some of these same compounds were isolated from white sugar. The presence of pyrazines in both wash water and white sugar suggests that the latter may acquire at least some odorants from wash water during the final wash step in the centrifugal.

Other substances implicated as sugar odorants are volatile fatty acids. These are produced during microbial infections and by sugar degradation during processing, and are responsible for the sour, rancid odors sometimes associated with sugar (8).

An extract of WCWW prepared using Optipore (conditions described above) was analyzed for organic acids by GC. In the separation profile shown in Figure 6 (upper panel), several small peaks were observed with retention times corresponding to those of volatile fatty acids. To confirm this, the sample was spiked with pairs of organic acid standards, then re-examined by GC. The lower panel of Figure 6 shows the separation profile of the extract after spiking with isobutyric and isovaleric acids. As shown, the peaks suspected of being organic acids were observed to increase in size. This approach was repeated using other organic acid pairs. In every case, the presumptive acid peaks were observed to become larger, indicating that the wash water contains acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids.

A sample of malodorous sugar was similarly screened for volatiles with Optipore. In this case, ~19 L of solution containing 40% w/v sucrose @ pH 6.22 were passed through an Optipore column (bed volume: 232 ml) at 30°C and a flow rate of ~780 ml/hr. After the entire solution had passed through the column, the resin was washed with 3.2 L of distilled water. Retained material was then eluted with 1.2 L of methanol at ambient temperature. Gas chromatography of the methanol extract (data not shown) revealed that the sugar sample contained acetic and isobutyric acids; traces of propionic and isovaleric acid may also have been present. The data

collectively demonstrate that volatile organic acids are present in wash water and white sugar. Organic acids have been found in beet sugar, beet molasses, and other beet factory process streams by several investigators (7,8,15,17). Thus, our findings are consistent with previously published data. Moreover, our observation that volatile acids are present in wash water and white sugar reinforces the notion that sugar may acquire some of its odor from wash water during the centrifugal washing step.

Up to this point, identification of organic acids was performed by GC analysis of Optipore and Empore extracts of test samples. Although effective, the approach was somewhat laborious and time consuming, requiring anywhere from 0.5 to 2 days to prepare samples for analysis. This necessitated an improvement in methodology. For this reason, the use of ion chromatography was explored.

The upper panel of Figure 7 shows the ion chromatographic (IC) profile of a series of organic acid standards. The lower panel shows a separation profile obtained with WCWW. By comparing the retention times of the sample peaks with those of the standards, it can be seen that acetic and isobutyric acid are present in wash water. This method was found to work well not only with a simple matrix such as water, but with sugar solutions as well. Figure 8 shows an IC profile obtained with a solution of production sugar (lower panel). Comparison with the standards in the upper panel shows that the sugar contains lactic acid plus several volatile fatty acids.

The data obtained by IC confirm the results obtained using adsorbents plus GC. More importantly, however, this method of analysis is simple, rapid, involves no extraction steps, and provides very high conductivimetric sensitivity. The speed, ease of use and sensitivity of the method give it excellent potential for rapid screening of sugar. Alternatively, it can be used to quickly monitor the effects of factory process changes on sugar quality.

Contract Laboratories

Beet sugar is sometimes described as having an earthy, musty odor. Several naturally-occurring substances have this odor (Figure 9). One is geosmin, or 1,10-trans-dimethyl-trans-9-decalol (6). This is a sesquiterpene derivative which is produced by Actinomycetes, blue-green bacteria, and some fungi (3,5,16). As little as 10-20 parts per trillion (ppt) of geosmin are detectable by the human nose (9). Another musty

odorant produced by bacteria is 2-methyl-isoborneol (MIB). This has a musty, camphorous aroma with an odor threshold of ~30 ppt.

Geosmin has been found in plant products such as navy beans and red table beets (1,2). Both compounds are often present in fish (e.g., catfish) to which they contribute off-odors and flavors (12,13). They are frequently found in ponds, lakes and rivers, and can also be present in municipal water supplies to which they impart muddy, musty odors and tastes. For this reason, metropolitan water companies have developed analytical techniques to measure these compounds in malodorous drinking water.

The odor caused by geosmin and MIB is similar to that reported in beet sugar, and, not surprisingly, they have been implicated as contributors to beet sugar odor. If they are present in sugar, a likely source could be centrifugal wash water. As was shown with pyrazines and organic acids, odorants in wash water may be deposited on sugar crystals during the wash cycle. To test this possibility, water samples from American Crystal Sugar factories were sent to a laboratory that routinely quantitates MIB and geosmin in municipal water supplies, namely the Philadelphia Suburban Water Company in Bryn Mawr, PA. This facility uses a procedure known as closed-loop stripping wherein volatiles are purged from water samples with helium, then adsorbed on a carbon trap. This is extracted with CS_2 and the extract analyzed by GC; identities of unknowns are confirmed by mass spectroscopy.

The results of these tests are shown in Table 3. Two different types of water samples were tested: white centrifugal wash water and condensates from the evaporators. MIB was found in only six of the samples, and in amounts that were well below threshold levels. On the other hand, geosmin was found in the majority of samples and, in most cases, was present in amounts that approached or exceeded the human olfactory threshold for this substance. Moreover, the geosmin levels were found to be especially high in 2nd effect condensate, a result that has been repeatedly confirmed by subsequent re-examination of water from this source (data not shown). This is significant in that 2nd effect condensate is sometimes used in making up water used for washing white sugar in the centrifugals. The data show that both geosmin and MIB can be present in factory process water; however, the concentrations of these substances vary from factory to factory and fluctuate during the campaign.

The presence of geosmin and MIB in wash water suggested that these compounds might also be in white sugar. For this reason, sugar samples were sent to

Montgomery Watson Labs in Pasadena, CA, for geosmin and MIB screening. None of the sugars were found to contain these substances. However, in early 1996, a sugar sample returned by a consumer because of an intense musty odor was also tested. Although geosmin was absent, the sugar was found to contain ~30 parts per trillion of MIB, a level sufficient to produce an objectionable odor.

In this study, we were unable to detect geosmin in our sugar. However, in 1994, Marsili et al (14) recovered from beet sugar a musty odorant identified as geosmin. The odorant was detected by GC with a sniffer port for olfactory evaluation and its identity as geosmin confirmed by mass spectroscopy. The geosmin content of the sugar was estimated at <25 parts per trillion which is equivalent to <25 grams of geosmin in 22 million cwt of sugar. Marsili's group concluded that combinations of geosmin plus acetic, butyric, and isovaleric acids produce the odor characteristic of beet sugar.

It is important to note that Marsili's data were obtained with raw, unconditioned sugar, whereas our sugar, which tested negative for geosmin, had been conditioned. This reinforces the importance of proper conditioning in sugar production. However, the available data indicate that geosmin and/or MIB may occasionally occur in beet sugar in levels sufficiently high to produce objectionable odors.

There are several possible sources of geosmin and MIB. Since they are synthesized by microbes found in agricultural soils, they could be produced by these organisms in soil adhering to the root surfaces. Also likely is the formation of geosmin and/or MIB by microbes on deteriorating beets under poor storage conditions. Once formed, these odorants could enter the factory as contaminants with the beets.

Another possibility is that geosmin is a normal component of the sugarbeet, and that traces of this material survive factory processing to accumulate in sugar during crystallization. Geosmin is known to be a natural component of red table beets (1,14); therefore, it is not unreasonable to speculate that it is present in sugarbeets.

Table 4 lists some of the compounds we have identified in beet factory process streams. Other volatiles were identified for us by the research staff at the Univ. of MN Food Science Dept. by GC-MS of samples obtained by liquid-liquid extractions with CH_2Cl_2 . Some of these are listed in Table 5.

Many of the compounds in Table 4 have been reported previously by other investigators. Most of these were detected in white sugar and centrifugal wash water. Some (e.g., geosmin and MIB) probably enter the factory with the beets, while others, such as organic acids and pyrazines, are probably formed during processing. These compounds may become included in sugar or be deposited on crystal surfaces during crystallization. Incomplete washing or washing the crystals with water containing these odorants could negatively impact sugar quality. Failure to adequately condition the sugar may also play a contributing role.

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Table 1. Causes of beet sugar odor.

1. Poor beet quality
2. Beet deterioration in storage
3. Improper beet washing
4. Incomplete removal of mother liquor from crystals
5. Odorants in wash water
6. Odorants in factory air
7. Microbial infections
8. High sugar moisture

Table 2. Properties of UOP'S "ABSCENTS" zeolite adsorbents.

"ABSCENTS"

UOP Corporation
Des Plaines, IL

1. Zeolite molecular sieve
2. Form: powder, beads, tile
3. Adsorbs odor from air & liquids
4. Regenerated with heat
5. Food grade product
6. Traps beet sugar odorants

Table 3. Some odorants identified in ACS factory process streams.

		Parts per Trillion		
Water	Date	Geosmin	MIB	
<u>Wash Water</u>				
Factory A	Feb '94	7.7	ND	
	Apr '94	7.9	ND	
	Dec '94	ND	ND	
Factory B	Feb '95	1.7	<5.0	
	Dec '94	41.3	ND	
	Jan '95	27.2	ND	
Factory C	Feb '94	1.7	ND	
	Dec '94	ND	ND	
	Feb '95	0.8	5.9	
Factory D	Feb '95	1.2	<5.0	
Factory E	Feb '95	1.7	<5.0	
<u>Evaporator Condensates</u>				
Factory A	#1	Dec '94	ND	ND
	#2	Dec '94	41.0	ND
	#3A	Dec '94	ND	ND
	#3B	Dec '94	ND	ND
	#4	Dec '94	ND	ND
	#2	Feb '95	44.9	5.4
Factory B	#2	Jan '95	59.5	ND
Factory C	#2	Feb '95	13.7	8.3
Factory D	#2	Feb '95	77.0	ND
Factory E	#2	Feb '95	14.0	<5.0

ND = Not Detected

Table 4. Some odorants identified in ACS factory process streams.

Odorant	Odor
2-Me-Pyrazine	Nutty, Burnt
2, 3-Di-Me-Pyrazine	"
2, 5-Di-Me-Pyrazine	"
2, 6-Di-Me-Pyrazine	"
2,3,5-Tri-Me-Pyrazine	"
Tetra-Me-Pyrazine	"
Acetic Acid	Sour, Rancid
Propionic Acid	"
Butyric Acid	"
Isobutyric Acid	"
Valeric Acid	"
Isovaleric Acid	"
Hexanoic Acid	"
Hexanal	Green, Grassy
Nonanal	"
Methyl-Isoborneol	Earthy, Musty
Geosmin	"

Table 5. Volatiles identified in ACS factory process streams by the University of Minnesota.

Compound	Source
4-Methylpyrimidine	Std Liquor
Cyclohexanol	"
2, 3-Octanedione	"
Furaneol	"
3-ET-2, 5-DiMe-Pyrazine	"
1, 3-Butanediol	"
2-Pyrrolidinone	"
2-Piperidinone	"
Para-Menthane	Wash Water (WCWW)
2-Phenyl-Ethanol	"
4-Ethyl-Phenol	"
α -Terpineol	"
Octanoic Acid	"
2-Cyclohexen-1-One	Std Liquor, WCWW
Dodecanoic Acid	"
Decanoic Acid	"
Vanillin	"

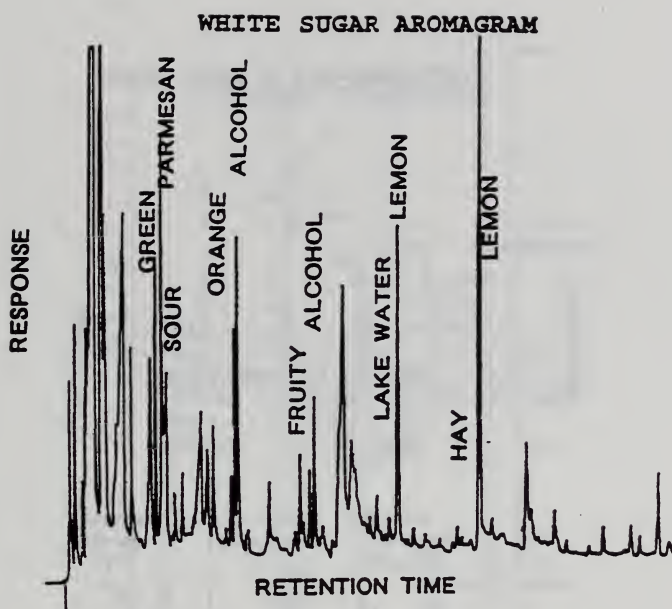


Figure 1. Aroma profile (aromagram) of volatiles released from sugar by the purge and trap method.

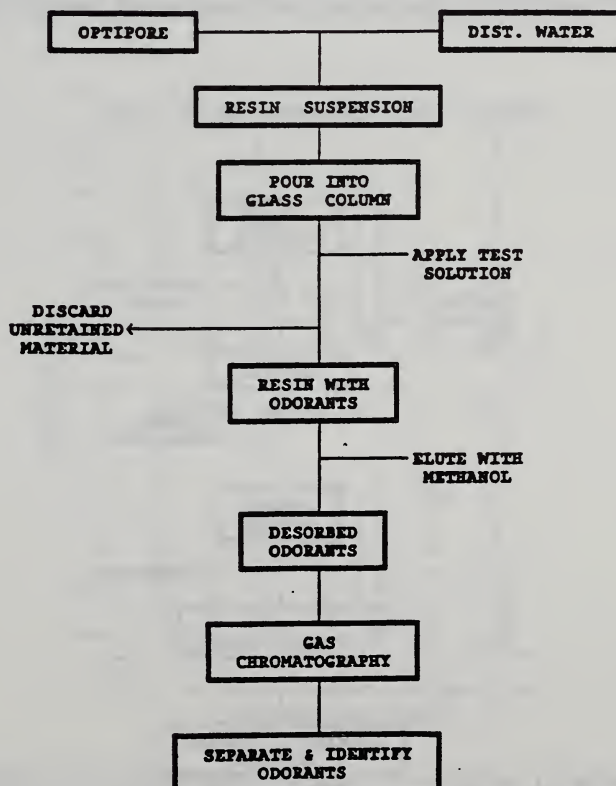


Figure 2. Schematic diagram for isolating sugar odorants using optipore.

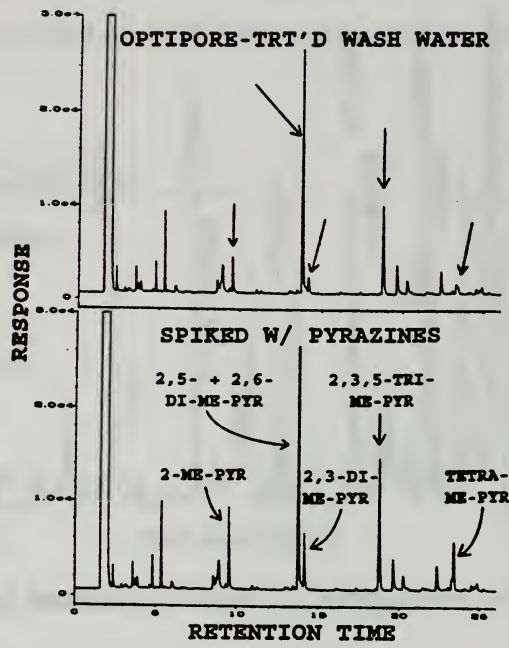


Figure 3. GC profile of pyrazines recovered from wash water using optipore resin.

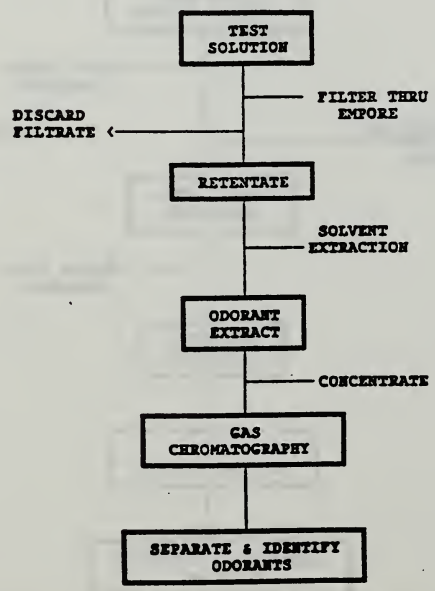


Figure 4. Schematic diagram for isolating sugar odorants using empore membranes.

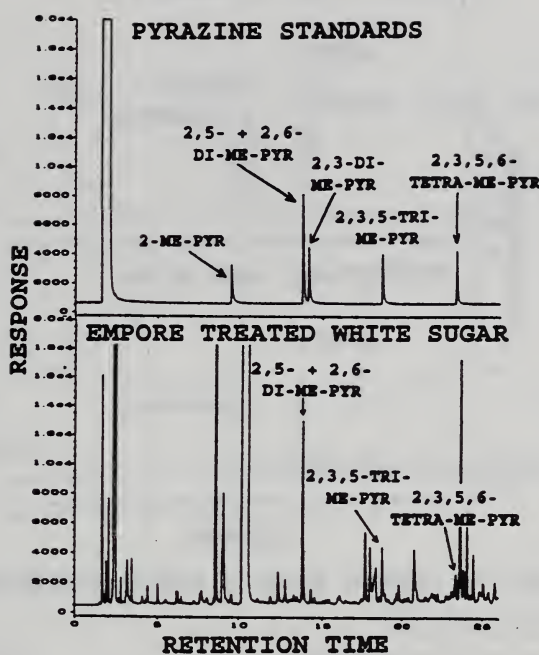


Figure 5. Identification of pyrazines in empore extract of white sugar.

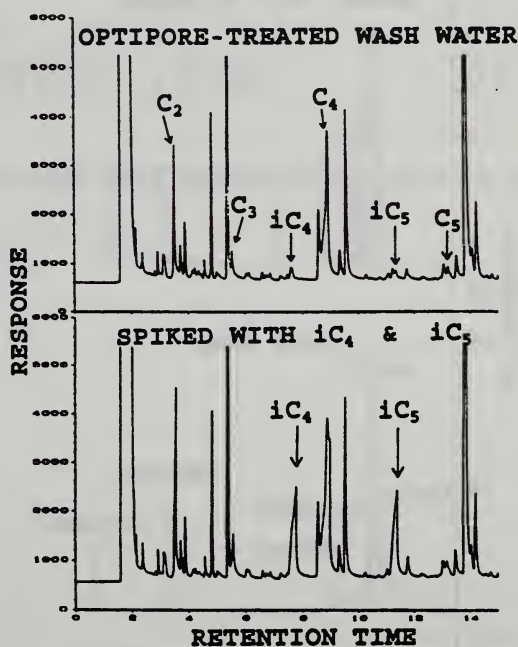


Figure 6. Identification of organic acids in optipore extract of white centrifugal wash water.

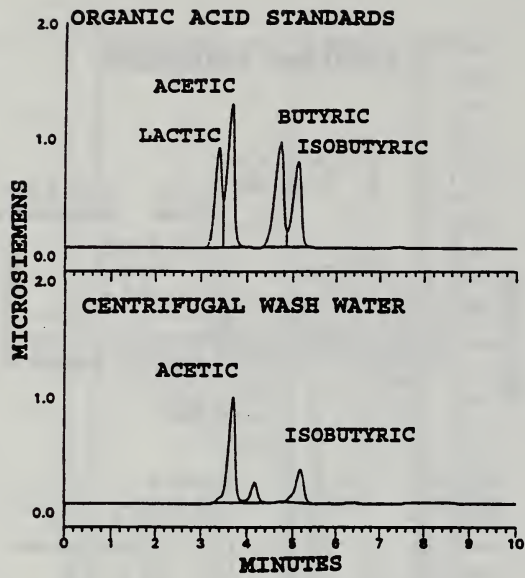


Figure 7. Identification of organic acids in white centrifugal wash water by ion chromatography.

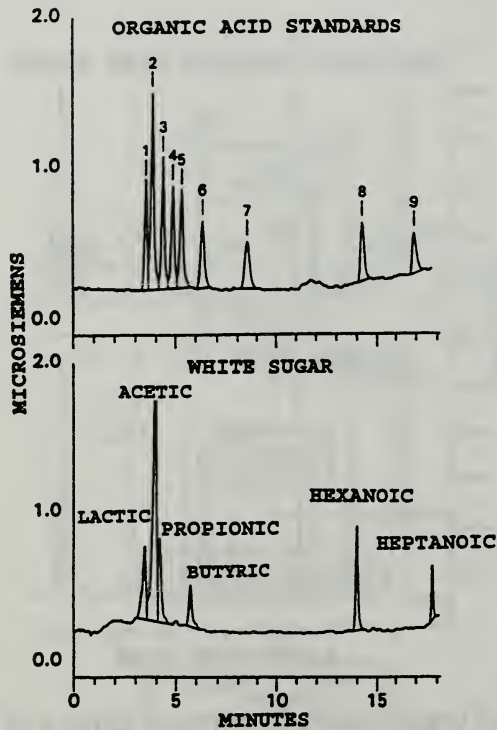
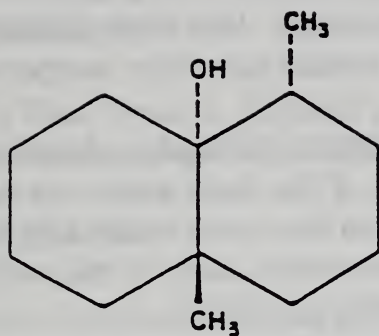
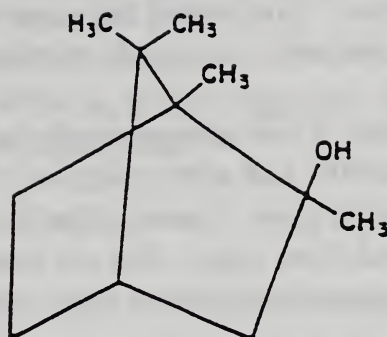


Figure 8. Identification of organic acids in white sugar by ion chromatography.



GEOSMIN



2-METHYL-ISOBORNEOL (MIB)

<u>ODORANT</u>	<u>OLFACTORY THRESHOLD (PPT)</u>
GEOSMIN	10
METHYL- ISOBORNEOL (MIB)	29

Figure 9. Structures and odor thresholds of naturally-occurring earthy, musty odorants.

DISCUSSION

Question: You treated the sugar with different materials. Was the liquid material, after treatment, completely odorless, or did it still contain some odor components?

Colonna: I can quote you only the result. We treated centrifugal wash water with Optipore™, and after treatment the strong odor of the wash water was almost completely gone. I assume that if odors are removed from wash water, they can be removed from sugar. But we have not done a systematic study of this, with test panels to evaluate treated sugar - that is an interesting possibility.

Question: One odor description that I did not see in your profile was the term "dirty socks" - a term that has been applied to sugar off-odor. Do you know what that component might be?

Colonna: That odor is usually attributed to isovaleric or isobutyric acid. We did find that in our wash water and in our sugar.

Comment: We can answer the earlier question about treating the wash water; and effectiveness of odor removal. Over the last several years, S.P.R.I. has published several papers (Clarke, M. A., E. J. Roberts, M. A. Godshall and X. M. Miranda. (1991). The polysaccharides of sugarbeet. *Proc., C.I.T.S.* pp. 421-332; Clarke, M. A., M. A. Godshall, R. S. Blanco and G. T. Perret. (1987). Beet sugar colorant: Recent studies. *Zuckerind.* 114: 709-713; Clarke, M. A. (1991). Pres. Irish Sugar Technical Conf., Killarney, Ireland) on the presence of organic acids in wash water and their contribution to odors in sugars. Working with several factories who had this odor problem, we suggested carbon treatment for wash water, particularly if second condensate was used for wash water. Many factories use this stream as wash water to recover the sugar but it can be a critical source of odor. Carbon treatment of wash water was successful. The organic acid background odor, and the "dirty socks" odor, were removed by carbon pad filters (and also by other more extensive carbon treatment).

Colonna: Yes, you will notice from Table 3 that second condensate is a stream that is really loaded with geosmin.

EXPERIENCE OF USING AMMONIUM BISULPHITE IN DIFFUSION AT BRITISH SUGAR

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ABSTRACT

Trials were carried out using ammonium bisulphite (ABS) as a biocide in diffusion at two British Sugar factories during the 1994/1995 campaign. It was demonstrated that bacterial activity during diffusion can be controlled effectively by maintaining the level of sulphur dioxide in raw juice at 150-200 ppm and the diffusion temperature above 70°C. There was a reduction in the pressed pulp dry substance which decreased from 29.1% when formalin was used in diffusion to 25.7% when ABS was substituted. There were also several incidents of high ash sugar (> 0.02%) resulting in white sugar returning for reprocessing. Analysis showed that the increase in ash was due to an increase in the concentrations of potassium, calcium and sulphate. It was postulated that this was due to the precipitation of a double salt of potassium and calcium sulphate, Kalussite.

INTRODUCTION

Thermophilic bacteria in continuous diffusers serve against good factory performance in a number of ways including:

- destruction of sucrose to yield acid (8)
- reduction of nitrate to nitrite, which leads to imidodisulphonate (IDS) formation in white sugar factories using sulphitation (2, 4).

The activity of thermophilic bacteria can be suppressed by raising the temperature or by the addition of chemical bacteriostats.

The usual factory practice is to employ a moderately high diffusion temperature of 68-73°C, in combination with formaldehyde, injected either continuously or by shock dosing. Complete suppression of the bacteria by heat alone is not practicable since a temperature of at least 80°C would be necessary (3).

Over the years, many sugar companies have tested a range of chemical agents to control bacterial activity in diffusion, e.g. peracetic acid (14), calcium hypochlorite (7), quaternary ammonium compounds (18) and hydrogen peroxide. In the past, British Sugar has evaluated sulphur dioxide in diffusion (9) with little success.

Recently, extensive research has been carried out to investigate the use of ammonium bisulphite and calcium bisulphite in diffusion (16, 17, 19). Bisulphites were added to both the head and the tail of the diffuser in trials carried out by Vaccari *et al* (16, 17). In addition to the successful control of the bacterial activity in diffusion, an improvement in the pressed pulp dry substance was also reported.

Trials were carried out at two British Sugar factories during the 1994/1995 campaign to assess the effects of using ammonium bisulphite in diffusion on the sugar process. The results are summarised and discussed in terms of diffuser sterility control, the effect on pressed pulp dry substance and white sugar quality in the present paper.

DIFFUSER STERILITY CONTROL

Diffuser sterility (less than 20 ppm nitrite and less than 20 ppm lactic acid in raw juice) was maintained throughout most of the trials at both Factory A (over a period of 80 days) and Factory B (over a period of 9 days). Continuous monitoring of nitrite, lactic acid and sulphur dioxide concentrations in raw juice showed that the first incident of infection, when the nitrite concentration in raw juice increased above 20 ppm, corresponded to a prolonged period when the sulphur dioxide concentration in raw juice fell below 120 ppm (Figure 1). This pattern was repeated for other incidents of infection.

Based upon all of the information gathered, the addition rate of ABS to both diffusion supply water and to the cossette belt was set to give a sulphur dioxide concentration in raw juice of 150-200 ppm to maintain sterility. The actual addition rates are given in Table 1.

In most cases of infection, the first sign was the appearance of the nitrite ion in raw juice. This agrees with the findings of Vaccari *et al* (16, 17) and Oldfield *et al* (10).

The effect of diffusion temperature on sterility is well documented (3). As the temperature decreases, the susceptibility to infection increases. Of the nine incidents of infection at Factory A, four were attributable to periods when the sulphur dioxide

concentration in raw juice was in excess of the target set above, but corresponded to temperatures in diffusion below 70°C. At Factory B, diffusion temperature was maintained at 70-72°C which, with a sulphur dioxide concentration in raw juice of 150-200 ppm, gave sterile diffusion over the entire trial period.

PRESSED PULP DRY SUBSTANCE

The use of bisulphite in diffusion has been reported to give an increase in pressed pulp dry substance (19). The use of the calcium salt, however, has often confused interpretation of the data as to whether it is the anion (bisulphite) or the cation (calcium) which is having the effect.

In laboratory studies carried out at British Sugar Technical Centre, a consistent, though statistically non significant reduction in pressed pulp dry substance was measured when ammonium bisulphite was added to diffusion supply water, even in the presence of calcium sulphate (15).

In the factory trials reported here, the addition of ammonium bisulphite gave a significant reduction in pressed pulp dry substance. At Factory A, there was a decrease in the composite pressed pulp dry substance from 29.1% to 25.7% (Table 2). At Factory B, an approximately 1% reduction in pressed pulp dry substance from 29.2% to 28.1% was measured compared to the period when formalin was used.

It is known that pressed pulp dry substance is affected by press speed; the higher the press speed, the lower the pressed pulp dry substance. Results obtained at the same press speed, showed that the pressed pulp dry substance decreased from 29.5% with formalin in diffusion to 27.0% with ABS. This suggests that there was a reduction in pulp pressability when ABS was used in diffusion. The reduction in pressability also increased demand for press capacity. To compensate this, the speed of the variable speed presses increased which further reduced the pressed pulp dry substance as shown in Table 2.

The press capacity, relative to throughput, was greater at factory B, which could explain the smaller reduction in pressed pulp dry substance.

Those factors known to affect pulp pressability include pH, diffusion temperature, pressing aid and degree of infection (11). In addition, it is known that pulp is susceptible to ion exchange and it is proposed that as all other conditions appeared

constant, the decrease in pressed pulp dry substance may be the result of an exchange of calcium ions on the pulp by ammonium ions with an associated decrease in pulp pressability. This hypothesis was not verified during the current study.

WHITE SUGAR QUALITY

During the trials, the main problem encountered in the sugar production process was a general increase in white sugar conductivity ash, with intermittent periods of very high ash.

The target white sugar conductivity ash at both factory A and factory B was 0.010% with an upper limit of 0.020%. When the conductivity ash exceeded 0.020%, the sugar was returned for reprocessing, with significant cost implications.

There were five incidents of high white sugar ash at factory A and two incidents at factory B with levels as high as 0.04% at factory A and 0.13% at factory B. Outside the trial periods, the ash was consistently below 0.010% at both factories. All incidents of high white sugar ash were attributed directly to the use of ammonium bisulphite in diffusion.

Analysis of Ash

Analysis of the high ash white sugar indicated a significant increase in the concentrations of potassium, calcium and sulphate ions. Changes in chloride, nitrate, magnesium and sodium were not significant. In most cases, potassium, calcium and sulphate ions accounted for about 80% of the increase in the conductivity ash (Table 3).

Factors Contributing Ash in White Sugar

Inclusion of mother liquor

Ash in white sugar may result from the inclusion of mother liquor and/or the precipitation/co-crystallisation of non-sugars during crystallisation. If inclusion was the cause of the high ash white sugar observed during the ABS trials, then a corresponding increase in solution colour would be predicted (1). Figure 2 shows the relationship between the conductivity ash and solution colour of the white sugar obtained during the ABS trial at factory A.

The results indicate that there was no increase in colour with increasing ash content, suggesting that the high ash was not due to the inclusion of mother liquor. It is also known that the inclusion of mother liquor gives rise to an increase in the internal water in the sucrose crystals (6). Four white sugar samples (two high ash, two normal ash) were analysed by Karl-Fisher titration and the results are shown in Table 4. There was no significant difference between the samples. The presence of a large number of irregular crystals is also an indication of inclusion (5). Microscopic examination showed that there did not appear to be an abnormally high number of irregular shape crystals in the high ash sugar, again suggesting that the high ash contents in white sugar were unlikely to be due to the inclusion of mother liquor.

Imidodisulphonate (IDS)

The reaction between nitrite and bisulphite in sugar solutions to produce long, needle-shaped crystals of imidodisulphonate is well documented (13). The appearance of IDS in white sugar is normally associated with an infection in the diffuser giving rise to elevated concentrations of nitrite. This then reacts with sulphur dioxide from the sulphitation stage to produce IDS.

In the trials described here, sulphur dioxide in raw juice derived from ABS could react with nitrite during short periods of infection according to the reaction described above.

The presence of IDS was assessed both microscopically, to detect the distinctive needle-shaped crystals and by a quantitative chemical test developed at British Sugar Research and Development in which the IDS was converted by hydrolysis with nitrous acid to sulphate. This was then measured by Dionex Chromatography. Examination of all high ash white sugar samples failed to detect significant concentrations of IDS (<5 ppm).

The precipitation of insoluble salts

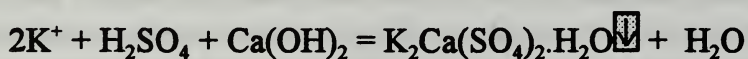
From the previous two subsections, it is concluded that the increased white sugar ash experienced during the ABS trials was not the result of mother liquor inclusion, nor IDS formation. It is, therefore, deduced that the increased ash results from the precipitation of insoluble salts.

The increase in white sugar ash (above) was shown to be due to an increase in potassium, calcium and sulphate concentration. These increases have been calculated as molar ratios. The results (Table 5) show a mean ratio of approximately 2:1:2 (K:Ca:SO₄), suggesting the salts involved could be potassium sulphate and calcium sulphate.

While the precipitation of calcium sulphate with its low solubility in water (0.21% w/v) might be predicted, potassium sulphate, with a much higher solubility of about 12% w/v is a less likely candidate.

It is, therefore, hypothesised that the ash is due to the precipitation of a double salt of potassium and calcium sulphate (KCS), also known as Kalussite (K₂Ca(SO₄)₂·H₂O). KCS has a solubility of 0.25% (w/v) in cold water, which is highly dependent on pH, i.e. soluble under acid but insoluble under alkali conditions. There is no information in the literature about the solubility of KCS in sugar solutions.

In experiments performed in British Sugar laboratories, a precipitate was obtained by progressively adding calcium hydroxide solution to potassium sulphate solution. Analysis showed that this precipitate had the same molar ratio of potassium, calcium and sulphate as KCS. Similar studies by a group of Japanese researchers (12) showed that when sulphuric acid and calcium hydroxide were added to molasses in order to reduce the potassium concentration, a double salt of potassium sulphate and calcium sulphate (KCS) was formed as shown by the following equation:



It appears that KCS is easily obtained in the presence of potassium, calcium and sulphate under alkaline conditions. The typical pH of standard liquor is 8.5, which from the experiments reported above would favour the precipitation of KCS.

Source of Increased Ash in White Sugar

Comparison of the analysis of standard liquor sampled during the production of low and high ash white sugar is shown in Table 6. The data shows a significant increase in both sulphate and calcium concentration in standard liquor during the high ash period.

In a sugar factory, sulphate in the process juices can be derived from a number of sources. These include the beet, sulphuric acid, gypsum (calcium sulphate) and sulphur dioxide into second carbonatation juice during sulphitation (a percentage of the sulphur dioxide is then oxidised in the process to sulphate).

During the ABS trial, a further source of sulphate was the oxidation of sulphite from ABS to sulphate. On average, the sulphate level in raw juice increased by 300 ppm (Table 7). Typically, 40% of the sulphate in raw juice is eliminated during juice purification. Thus, the 300 ppm increase in raw juice sulphate would result in a net increase of 180 ppm sulphate in the carbonated juice during the ABS trials. During evaporation, thin juice is concentrated by about 4.2 times and thus, an increase in sulphate concentration from 180 to 760 ppm would be predicted. This is equivalent to 1100 ppm on sugar and can, therefore, account for the increased concentration as shown in Table 7.

The elevated concentration of calcium in standard liquor can be explained by the relatively higher solubility of calcium sulphate (0.21% w/v) than calcium carbonate (0.002% w/v), which consequently passes through juice purification into the sugar end.

In summary, the increase in raw juice sulphate resulted in significant increases in standard liquor sulphate and calcium concentration, and consequently ash in white sugar.

CONCLUSIONS

1. Diffuser sterility was generally maintained with 150-200 ppm residual sulphur dioxide in raw juice and a diffusion temperature of 70-72°C.
2. When ammonium bisulphite was added to the process, there was a reduction in the pressed pulp dry substance of up to 3.4%.
3. There were five incidents of high ash white sugar (>0.02%) over a period of 80 days at factory A and two incidents over a period of 9 days at factory B. Analysis showed that the increase in ash was due to an increase in the concentrations of sulphate, calcium and potassium.

4. It is hypothesised that the increase in conductivity ash in white sugar is due to the precipitation of a double salt of potassium and calcium sulphate (Kalussite).

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Table 1. ABS addition and measured SO₂ levels in factory juice.

	ABS Addition Rate (g SO ₂ /t Beet)	Measured SO ₂ (ppm)
Diffusion Supply Water	300	200-250
Cossette Belt	245	150-200*

*Measured sulphur dioxide concentration in raw juice

Table 2. Change in pressed pulp dry substances.

	Factory A		Factory B	
	Pre-trial	Trial	Pre-trial	Trial
Pressed Pulp Ex-fixed Speed Press	29.5%	27.0%	-	-
Composite pulp	29.1%	25.7%	29.2%	28.1%

Table 3. Composition of white sugar ash.

Conductivity Ash (%)	Potassium (ppm)	Calcium (ppm)	Sulphate (ppm)	% Ash increase due to K, Ca, SO ₄
0.008*	26	3	8	-
0.026	49.5	21.5	98	83
0.039	102	40	147	81
0.042	96	38	193	85
0.044	70	35	165	65
0.129	340	144	556	83

* Typical pre-trial analysis

Table 4. Total moisture in white sugar samples.

Sample	Moisture (%)
Pre-trial sugar	0.033
Pre-trial sugar	0.034
Trial sugar	0.033
Trial sugar	0.033

Table 5. Molar ratio showing increase in calcium, potassium and sulphate during ABS trials.

Conductivity, Ash (%)	Potassium	Calcium	Sulphate
0.026	1.3	1.0	2.0
0.039	2.1	1.0	1.6
0.042	2.1	1.0	2.2
0.044	1.4	1.0	2.0
0.129	2.3	1.0	1.6
Mean	1.8	1.0	1.9

Table 6. Potassium, calcium and sulphate contents in standard liquor (based on sugar) during high ash and normal ash periods.

	Normal Ash Period	High Ash Period	Statistical Significance
Potassium (ppm)	6700	7000	No
Calcium (ppm)	340	400	Yes*
Sulphate (ppm)	3000	3400	Yes**

*95% confidence level

**99.99% confidence level

Table 7. Raw juice and standard liquor concentration before and during the ABS trials.

	Pre-ABS trial	ABS trial
Raw Juice Sulphate (ppm)*	550	850
Standard Liquor Sulphate (ppm)**	2100	3300

* As is
**Based on sugar

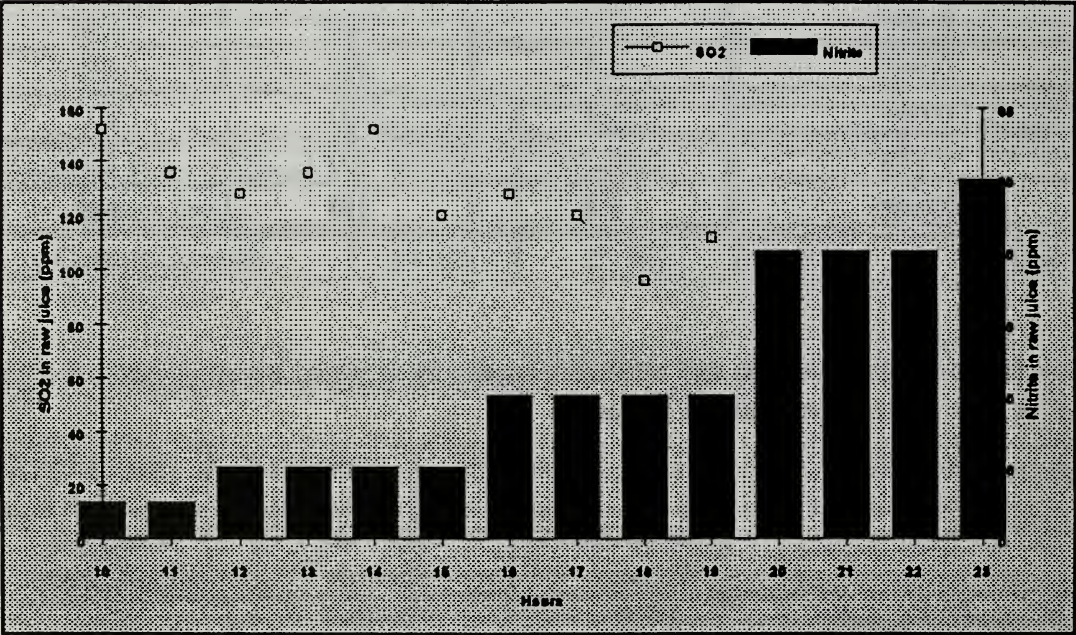


Figure 1. The effect of sulphur dioxide on nitrite concentration in raw juice.

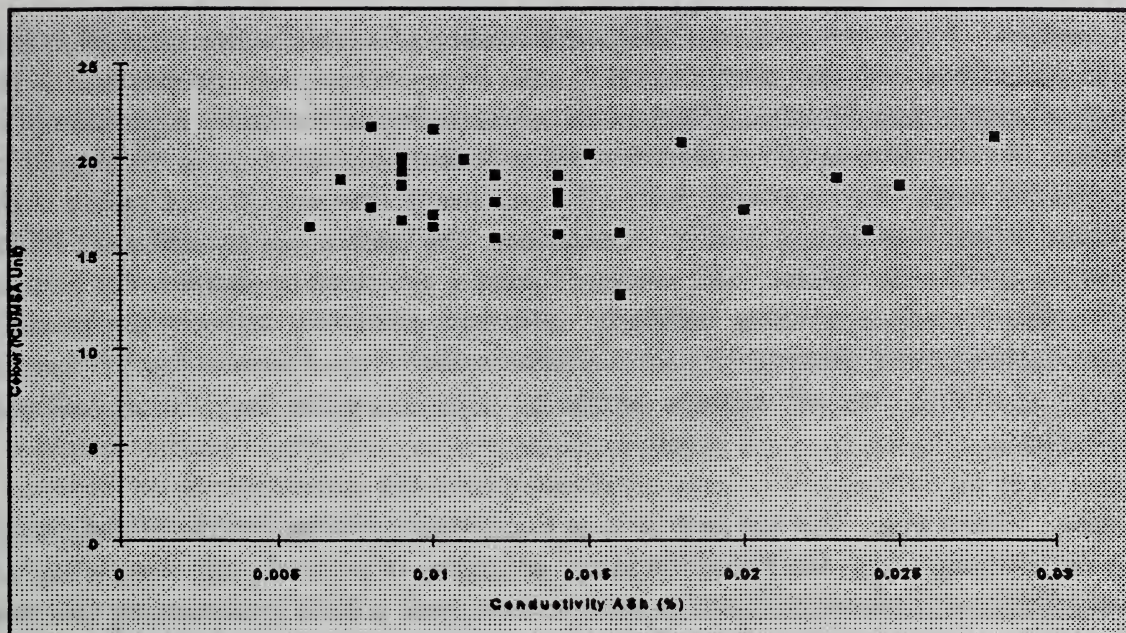


Figure 2. The relationship between conductivity ash and ICUSMA solution colour of white sugar.

DISCUSSION

Question: I agree with you regarding the formation of double sulfate salts of sodium and potassium, and their contribution to ash. In my experience, independent of the use of ammonium bisulfite, when the factory is using an unusually high amount of sulfite, or of calcium sulfate in diffusion, there is always an increase in the amount of ash in the sugar. It is probably due to the increase in concentration of sulfate in the juices; when its solubility product is exceeded, the double salt precipitates. If you wish to sterilize the diffuser with calcium bisulfite, you need to decrease the amount of sulfuric acid or calcium sulfate in diffusion. Possibly this can be partially substituted (for pulp purposes) by the additional sulfate formed from sulfite. The total level of sulfate from all sources should be kept below the level for double salt formation.

Tian: Thank you. The ash level was an occasional problem, but the real difficulty in the campaign was the reduction in dry substance in pressed pulp, even when we used the same amount of bisulfite as of sulfate press aid. It is interesting that you mention calcium bisulfite. We did not use calcium bisulfite, principally because, in initial laboratory experiments, we found it very difficult to control the pH in that system. Calcium bisulfite does not really exist as a chemical entity - it is a mixture of calcium sulfite dissolved in sulfite solution. This has a very low pH and leads to problems with corrosion.

Question: It is refreshing to hear results that are not always good, so that other people can avoid making the same errors. You showed two adverse effects; the dry substance yield was lower, and the sugar quality was deteriorated. Two questions: (1) Have you measured lactic acid, because the pulp press manufacturers demand at least 200 ppm lactic acid? (2) After these experiences, do you intend to continue the use of ammonium bisulfite?

Tian: These were trials to test the use of ABS. The answer is no - the factories will not use ABS. Not unless we can find a way to control the ash level and restore the pulp dry solids.

With regard to lactic acid, the use of ABS was in part designed to control lactic acid production to low levels.

PROCESS TITRATOR WORKING ON LIMED AND CARBONATED JUICES

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ABSTRACT

The determination of alkalinity, total alkalinity and calcium salts are vital measurements in the sugar factory. These measurements have traditionally been carried out as manual titrations performed by the operators. Today a number of automatic process titrators are commercially available, but they are all best suited for handling rather clean juices like filtrates and thin juices. Juices with a lot of lime and many particles cannot be handled.

In this perspective Danisco Sugar has developed a robust and automatic process titrator for use on all relevant juices in the purification step and especially on limed raw juice.

The two main parts of the process titrator are a robust juice sampling device and a titration module. The titration module is controlled by a standard autoburette combined with a non-contacting color measurement for detection of the titration point. The non-contacting color measurement ensures that scaling of the titration point detector will not happen. The titration curve is sampled in a computer, where the correct titration point and the final alkalinity, total alkalinity or calcium salts are calculated.

One of the objectives of the process titrator is that it must be able to run steadily without maintenance for a period of 7 days, where refill of chemicals and minor cleaning are allowed. The sampling device and the titration module has proven its robustness on limed raw juice as well as on the other juices in the purification step. The process titrator has now been running for 2 campaigns in Danish factories with good results.

INTRODUCTION

In order to create a fully automated juice purification step it is necessary to have automatic measurements of alkalinity, total alkalinity and calcium salts in the relevant process streams in the sugar factory. These process streams range from cold and hot limed juices full of particles to the clear thin juice. A number of automatic process titrators are commercially available, but they are best suited for handling rather clean juices like filtrates and thin juices. Juices with a lot of lime and many particles cannot be handled.

Since the process titrator shall be able to take over the manual titrations traditionally performed by the operators, it is vital that it is robust and can run steadily without maintenance for a longer period. In a sugar factory such a period would preferably be about 7 days.

A presentation of our first prototype of the process titrator was given at the CITS Committee Meeting in Peterborough 1993 (1). At that occasion several implications for further work were given. Since 1993 we have pursued the goals set up there, and this paper will give details of this development. The instrument, at that time called the alkalinity-meter, is now able due to three versions of software to perform several titrations on different juices.

METHODS AND MATERIALS

Basic working principle of the process titrator

The entire process titrator installation is shown in Figure 1. The two main parts of the process titrator are a robust juice sampling device and a titration module, shown in Figure 2. The titration module is controlled by a standard autoburette combined with a non-contacting color measurement for detection of the titration point. The non-contacting color measurement ensures that scaling of the titration point detector will not happen. The titration curve is sampled in a computer, where the correct titration point and the final alkalinity, total alkalinity or calcium salts are calculated.

In order to monitor all relevant juices in the juice purification step the process titration is divided into 3 separate, but mechanically identical, titrators.

Titration 1 performs analyses of alkalinity and total alkalinity in cold and hot limed juices. The alkalinities range from 0.1 to 1.5% (w/w) CaO, and the total alkalinities range from 1.0 to 4.0% (w/w) CaO.

Titration 2 performs analyses of alkalinity in hot saturated, filtered juices, where the alkalinities are in the range 0.0-0.2% (w/w) CaO.

Titration 3 performs analyses of free calcium (hardness) in hot filtered juices, the 2. carbonation filtrate and the thin juice. These are in the range 0.001-0.01% (w/w) CaO.

The three titrators are mechanically completely alike and differ only in the software and the reagents applied. Three titrators are necessary because of the inherent risk of sample cross contamination. With the large difference in ranges of the parameters to be determined, cross contamination would spoil the results.

The titrators are built from standard components and installed in a stainless steel cabinet of height 1.9 m, width 0.7 m, and depth 0.5 m. The cabinet contains three compartments (electronics, mechanics and reagents). Sample changing is on the left side of the cabinet.

The principle of operation for alkalinity and total alkalinity determination is as follows:

- 1) The juice to be analysed is pumped from a liquid flow of low pressure preferably just before an outlet to atmospheric pressure (overflow) with the use of a peristaltic pump, which is run in reverse in between sampling.
- 2) The juice is pumped to an overflow bin (sample changer of carousel type) from where it is sampled by a measuring cup (10 ml).
- 3) The juice is transferred to a small titration bowl.
- 4) The sample is diluted by adding water which at the same time cleans the measuring cup of sample residues.
- 5) The necessary indicator (phenolphthalein) is added by a piston pump, and the colour is measured.

- 6) The titration by acid from an autoburette is started, and the colour of the liquid (R, G and B-signals) is measured during the titration period every 0.5 seconds.
- 7) At the end of the titration which is carried on beyond the end point, the end point is determined by using a simple patented algorithm (2) on the color data. The corresponding analysis value of interest is calculated, and the titration bowl is emptied by a water jet pump and cleaned twice by flushing with water.

In the case where both alkalinity and total alkalinity values are desired, the two values are determined consecutively on the same sample by doing a second titration with a second indicator (methyl orange) after titration to beyond the first end point.

Calcium hardness is determined by using eriochromium black T indicator and EDTA solution as the titrant.

Major developments

A major point of concern has been the sampling process which due to the occurrence of sand and small beet particles must be very rugged. This problem was solved by using a peristaltic pump. To avoid particle build up in the tubes, the pump had to be run in reverse between sampling. All kind of valves in contact with the fluid to be sampled has been eliminated.

Since the first prototype the controller has been changed from a PC to a PLC (Siemens 95U with OP 25 display). The major reasons for this are:

- * The PLC is more robust in a factory environment.
- * The PLC has a longer life cycle than the PC due to the very rapid development seen for PCs meaning easier follow up.
- * PLCs can now be equipped with graphics displays to make it almost as user friendly as a good PC-program.
- * The memory capacity of a PLC is now large enough to accomodate the necessary programs which can be made in the PC environment.

The programs to control the titrators have been made more user friendly in that all parameters can be keyed in by the operator (different operator levels are possible). First of all the kind of juices and the analyses to be performed can be changed at will. Secondly, all parameters for a specific analysis can be changed (alarm limits, titration rates, acid strength, etc).

The instrument is fine tuned by comparing with laboratory titrations, and the exact calibration is keyed into the titrator.

RESULTS AND DISCUSSION

Titration 1 in its final form has been running at the Gørlev sugar factory during the 1995 campaign. Except for minor mechanical problems which are now solved, the titrator has carried out determinations of alkalinity and total alkalinity in cold prelimed juice, cold medium limed juice and hot main limed juice, in all 6 analyses every 30 minutes. A 5 minute check-up every day is advisable, but the instrument has in periods been run for 1 week without service.

Examples of titrations compared with titrations carried out by hand are given in Figure 3 and Figure 4.

The repeatability of the instrument is better than 5% which is adequate for most purposes. The accuracy of the instrument can be kept within 10% of the laboratory value.

CONCLUSION

Titration 1, performing analyses of alkalinity and total alkalinity in cold and hot limed juices, has proven to work well in the sugar factory for a whole campaign.

The titrators 2 and 3 will be built this summer and installed at the Gørlev factory together with titration 1 before the 1996 campaign. The titrators will act as transmitters and the signals will be used as input to a new completely automatic juice purification control in Gørlev.

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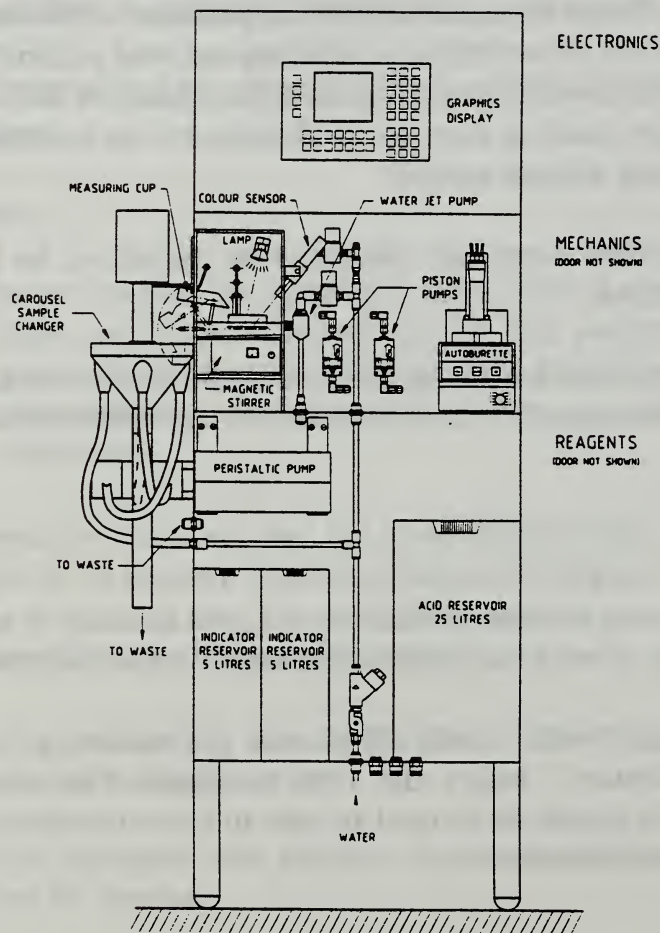


Figure 1. The process titrator installation.

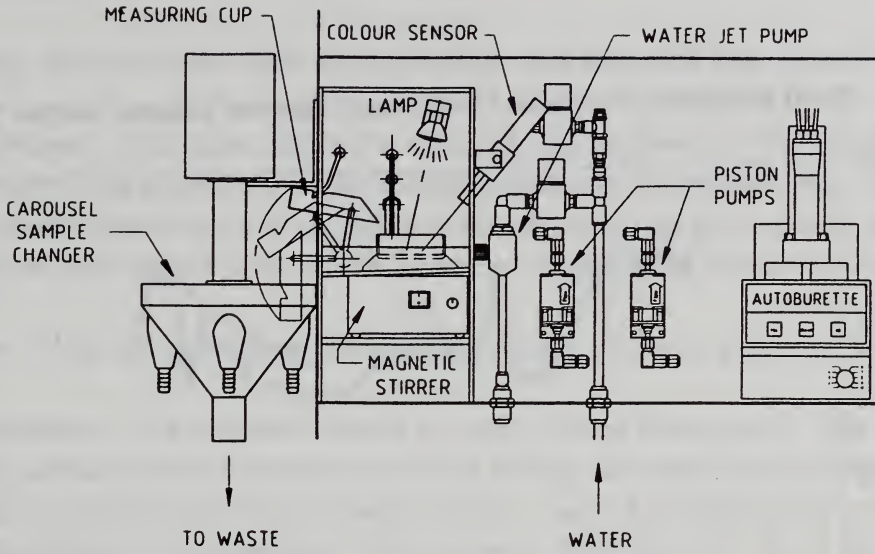


Figure 2. The basic mechanical parts of the process titrator.

Alkalinity, Process Titrator and Manual Values, Gørlev, 1995

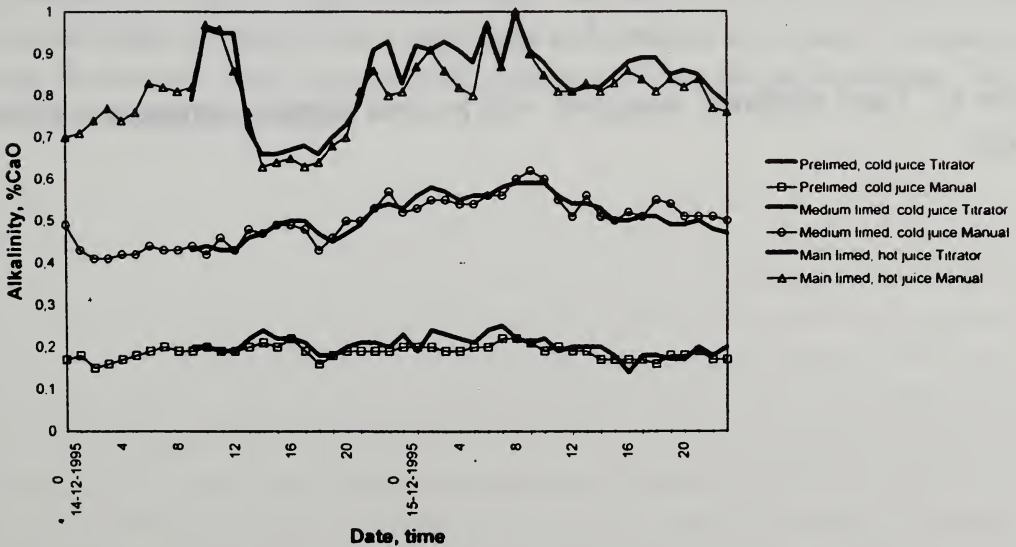


Figure 3. Alkalinity measured with process titrator compared with manual results.

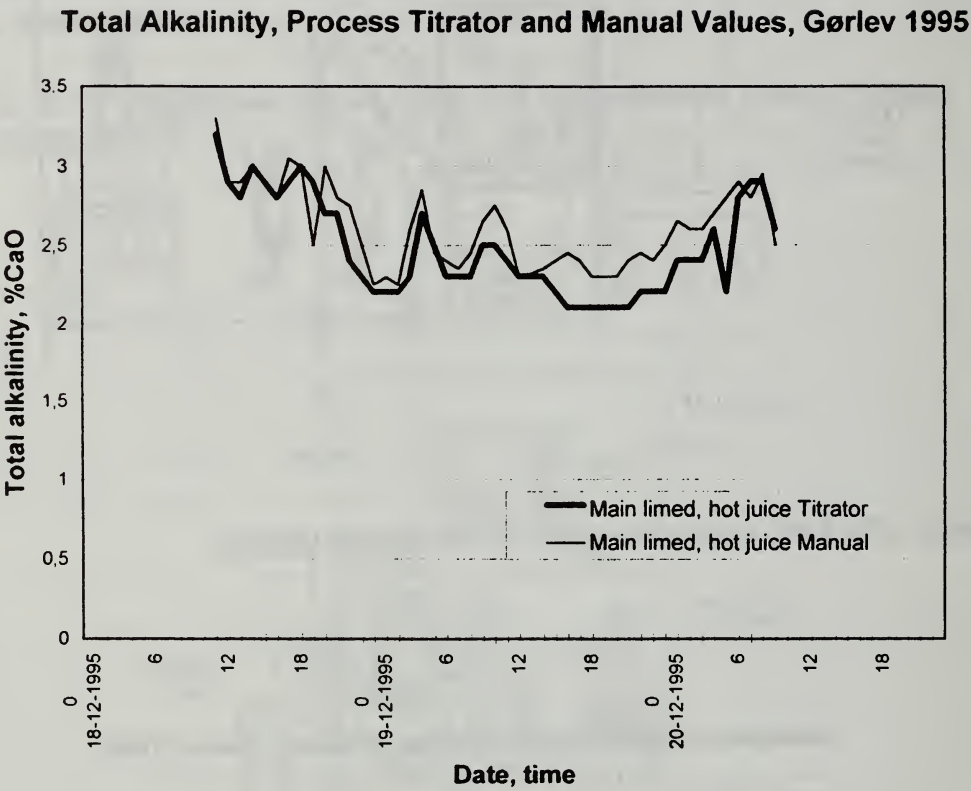


Figure 4. Total alkalinity measured with process titrator compared with manual results.

DISCUSSION

Question: Is this titrator now on the market, and if so, for how much?

Gudmundsen: We have decided not to sell this ourselves. We intend to find a company to sell the product, and take care of maintenance, services, etc. So, it is not available on the market yet, but should be in future. As to the cost, based on what the parts cost us, the titrator cost should be in the range of \$10,000-\$15,000.

Question: Why did you choose color to determine your end point, rather than pH?

Gudmundsen: We wanted to have a tough, robust instrument. We planned to avoid all contact with the sample, to avoid scaling and mechanical problems. For those reasons, we chose the color measurement. There are alternatives - you can use the conductimetric measurement, for example, but then you have to clean and standardize the detector - it will change with time. We chose color so that the processor could run for at least one week without any maintenance. That has succeeded.

Question: What is the cycle time? How frequently can the titrator make a measurement?

Gudmundsen: In Görlev Sugar factory, where we measured all six streams: alkalinity and total alkalinity of the pre-lime, main lime and medium lime juices, we use cycles of about 30 minutes. Every hour we had two sets of data for all six values. So, it is about five minutes per analysis.

MODEL STUDIES TO CHARACTERIZE THE THERMAL DEGRADATION OF CONCENTRATED AQUEOUS SOLUTIONS OF SUCROSE UNDER CONSTANT pH CONDITIONS

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ABSTRACT

The thermal degradation of sucrose leads to many reaction products that can reduce the efficiency of sugar mill and refinery processes, and decrease sucrose yield. Characterization of sucrose degradation under modelled industrial processing conditions will underpin further technological improvements. Effects of constant reaction pH on sucrose degradation were investigated using model systems (100°C; 65 Brix; N₂; 0.05-3M NaOH titrant), with the use of an auto-titrator. Reaction pH values ranged from pH 4.4 to 10.45. Polarimetry (at 589 and 880nm) and ion chromatography with integrated pulsed amperometric detection (IC-IPAD) techniques were used to monitor the kinetics of sucrose degradation and first-order reaction constants were calculated. Sucrose degradation, color and total organic acid formation were all markedly influenced by pH and temperature. Organic acid formation was detected using ion chromatography with conductivity detection. Separation and identification of oligomeric products, formed during acid and alkaline degradation of sucrose, using a developed IC-IPAD method with a NaOAc gradient in NaOH, are discussed.

INTRODUCTION

The thermal degradation of sucrose, and the formation of colored and non-colored products, is of considerable importance to the sugar industry; not only from the standpoint of valuable sugar losses, but from the reduction in unit process efficiencies and end product quality. Consequently, the degradation of sugars in aqueous acid and alkaline solutions has been widely studied and been the subject of several comprehensive reviews (1,2,3), with the latest review (3) relating the current understanding of the mechanisms of sucrose degradation to product loss. Despite the previous and continued interest in sucrose degradation, comparison of literature data is difficult. Most investigators have chosen to study sucrose degradation under various reaction conditions which were too far removed from the conditions in the

factory or refinery. For example, much work has been accomplished on dilute solutions and extrapolation to concentrated solutions is questionable. However, there has not been a systematic screening of the influence of reaction variables on the course of sucrose thermal degradation, under industrial processing conditions, particularly on high Brix solutions commonly found in the sugar industry.

The rate and course of the acid and alkaline degradation of sucrose can be influenced by several reaction parameters, such as the H^+ and OH^- concentration of the aqueous solution respectively, reaction temperature, presence of salts, concentration of sucrose and monosaccharides therein, and nature and pressure of the gas applied. The present knowledge of the role of each parameter is relatively small, but the development of highly accurate techniques such as ion chromatography, as described in this study, will increase knowledge. pH is known to be critical but reaction pH naturally decreases with time and, therefore, a full elucidation of pH effects has not been ascertained. To overcome this problem, some researchers (4) have studied sucrose degradation in buffered solutions. However, this introduces another parameter of salt cations which have recently (5,6,7) been shown to unequivocally catalyze degradation in 65 Brix sucrose solutions.

The aim of this study was, therefore, to undertake model thermal degradation reactions of concentrated aqueous sucrose solutions (~65 Brix), under a wide range of constant pH conditions [to simulate various industrial unit process conditions] to elucidate the role of pH and temperature. Model reactions were kept at constant pH using an auto-titrator, rather than a buffer, to reduce interfering ion effects.

EXPERIMENTAL

Constant pH Model Systems. Constant pH reactions were undertaken in a 1 L jacketed reaction vessel equipped with an Ingold™ combination pH electrode connected to a Metrohm 716 DMS Titrino autotitrator, a magnetic stirrer, a sampling syringe, pure nitrogen gas inlet and outlets and a dosing tube for adding titrant (M NaOH). The auto-titrator was calibrated with two different dilute pH (7 and 10) buffers at the reaction temperature, unless otherwise specified. Sample solutions (65 Brix; 800 mL) were first dissolved at room temperature and then allowed to equilibrate at the reaction temperature before the initial pH was adjusted to the reaction pH and the auto-titrator started. Reaction samples (~5 mL) were removed every 30 min and allowed to cool before weight dilutions were undertaken for analyses.

Brix. Brix was measured using an AbbeTM Refractometer.

Polarimetry. Pol of diluted samples was measured at 589 and 880 nm in a 20 cm cell, on a RudolphTM Autopol 880 Polarimeter, calibrated in ISS (Z scale) at 20 °C.

Color. Color was measured as the absorbance at 420 nm and calculated according to the official ICUMSA method (GS1-7).

Ion Chromatography (IC) - Mono and Disaccharides. Sucrose, glucose and fructose were separated on a DionexTM CarboPac PA-1 analytical anion exchange column at room temperature. Flow rate = 1.0 ml/min. Column eluant conditions were: 16mM NaOH isocratic (inject; 0.0-2.0 min), a gradient of 16-160mM NaOH (2.0-35.0 min), followed by isocratic 200mM NaOH (35.1-37.0 min), and return to 16mM NaOH (37.1-50.0 min) to re-equilibrate the column. Carbohydrates were detected using integrated pulsed amperometric detection (IPAD). The detector was equipped with Au working and Ag/AgCl reference electrodes, operating with the following working electrode pulse potentials and durations: $E_1 = +0.05$ V ($t_0 = 0.00$ sec), $E_2 = 0.05$ V ($t_1 = 0.42$ sec), $E_3 = +0.75$ V ($t_3 = 0.43$ sec), $E_4 = +0.75$ V ($t_4 = 0.60$ sec), $E_5 = -0.15$ V ($t_5 = 0.61$ sec), $E_6 = -0.15$ V ($t_6 = 0.96$ sec). Duration of the IPAD integration interval was 0.2-0.4 sec. Using a Spectra-PhysicsTM SP8880 autoinjector and Dionex AI-450 chromatography software, duplicate runs were accumulated of diluted samples and 10 levels of standards (glucosamine-HCl [internal standard], glucose, fructose, sucrose, raffinose and stachyose).

Ion Chromatography (IC) - Oligosaccharides. Oligosaccharide degradation products (mainly 2-12 degrees of polymerization) were separated on a Dionex CarboPac PA-1 column, using a NaOAc gradient at room temperature. Flow rate = 1.0ml/min. Column eluant conditions were: 100mM NaOH isocratic (0.0-1.1min; inject 1.0min), a gradient of 0 to 300mM NaOAc in 100mM NaOH (1.1-40.0min), and return to 100mM NaOH (40.1-45.0min) to re-equilibrate the column. IPAD and electrode settings were as described above.

Ion Chromatography (IC) - Organic Acids. Organic acids were separated on Dionex Ionpac AS11 analytical column at room temperature; an ATC-1 anion trap column was incorporated in the system. Eluant flow rate = 2.0 mL/min. Column eluant conditions were: isocratic 0.5mM NaOH for 7 min to equilibrate the columns, then isocratic 0.5mM NaOH (inject; 0.0-2.5 min), a gradient of 0.5 to 50mM NaOH (2.5-

6.0 min), followed by a gradient of 5.0 to 38.25mM NaOH (6.0-18.0 min). Organic acids were detected using conductivity detection with the use of an auto-suppression recycle mode using 50mN H_2SO_4 as the regenerant and an anion micromembrane AMMS suppressor. Organic acid standards (including aconitic, lactic, malic, formic, levulinic and acetic acids) were injected separately onto the column to obtain peak retention times for identification purposes.

Kinetic analyses. Kinetic raw data obtained from polarimetry and IC-IPAD, were Brix adjusted, and used to calculate first-order rate constants by use of a linear, least squares computing method.

RESULTS AND DISCUSSION

Constant pH Model Systems - Auto-titration Conditions.

The autotitrator system used to undertake the constant pH model reactions is shown in Figure 1. For each individual experiment, it was necessary to undertake preliminary experiments to optimize the system for auto-titration. This involved optimizing the correct titrant concentration and minimum dosing rate (see Table 1); both parameters are critical in preventing pH overshoot problems and ensuring adequate titration rates. pH overshoot problems are particularly problematic with high Brix sucrose solutions because of the low diffusability of the titrant.

It was impossible to initially adjust and maintain a $\text{pH} > 9.25$, without adversely affecting the reaction Brix. This is due to the high dissociation of water and sucrose at 100°C . [For example, a simple titration of 65 Brix sucrose at 100°C , showed that 28 mLs of 3M NaOH was required to obtain a pH of 10.55.] An increase in titrant concentration above 3M was not possible due to increased viscosity effects impeding the auto-titrator operation. Consequently, to undertake a reaction at constant pH 10.1, a 65 Brix sucrose solution had to be first dissolved in 500mM NaOH, rather than water. On pre-reaction equilibration at 100°C the pH of the solution was 10.1 - hence the reaction pH. To achieve a reaction $\text{pH} > 10.1$, a 70 Brix sucrose solution had to be dissolved in water at room temperature (>70 Brix sucrose solutions will not fully dissolve at room temperature and increased temperatures would only induce premature degradation; furthermore, dissolving a 65 Brix solution in >500mM NaOH induced alkaline degradation at room temperatures). The 70 Brix solution was allowed to equilibrate at 100°C and the initial pH adjusted by adding sufficient drops of concentrated (50% w/w) NaOH to adjust the pH to 10.45; this caused the Brix to

decrease to an acceptable level. Within the parameters of the experimental design and titrator capability, a $\text{pH} > 10.45$ was not achievable.

Constant pH Effects: Color and Total Organic Acid Formation

A plot of ICU_{420} color after 8 h reaction time against constant reaction pH is illustrated in Figure 2 and gave an excellent second order polynomial fit ($r^2 = .945$). Minimum color formation was at pH 5.45. The more highly acidic conditions at pH 4.4 will have induced slightly higher color formation. Color formed from alkali degradation reactions became predominant at $\text{pHs} > \sim 7.5$. A very similar curve fit was observed when titrant added was plotted against constant reaction pH (see Figure 3). This highly suggests that color formation is concomitant with total organic acid formation, as titrant added is an indirect measurement of total formation of organic acid degradation products. De Bruijn (8) observed that there appeared to be a direct relationship between color formation from the alkaline degradation of monosaccharides and $>\text{C}_6$ organic acid formation. A further plot of titrant added versus color formation (see Figure 4) illustrates that at higher alkaline pHs , i.e. > 9.25 , there was an unexpectedly marked increase in color and organic acid formation, reflecting the strong influence of OH^- concentration.

Constant pH Effects: Sucrose Degradation Kinetics.

Sucrose degradation was monitored using two techniques: polarimetry and IC-IPAD with a NaOH gradient (see EXPERIMENTAL section), the latter is an accurate and sensitive technique as it measures sucrose directly. As a plot of log of sucrose remaining against time was linear, first-order kinetic constants were calculated from the pol and IC-IPAD data and are shown in Table 2. The IC-IPAD kinetic constants indicate similar rates and, therefore, extents of sucrose degradation occurred at both extremes of pH studied, even though much higher color formation occurred at the extreme alkaline conditions (see Figure 4). This further suggests that color formation under alkaline conditions is a result of subsequent degradation of initial sucrose degradation products and/or degradation of trace amounts of monosaccharides present in the analytical sucrose used. Within experimental error, under alkaline degradation conditions pol generally did not decline across reaction time even though color formed and degradation occurred (an example is shown in Figure 5a). Formation of degradation products with a positive pol will have suppressed the change in pol. Therefore, polarimetry is not a viable technique for measuring sucrose degradation /losses at the levels that occur in factory and refinery unit processes

where pH is alkaline, e.g., in carbonation. Under acid degradation conditions (pHs 4.4-6.45) pol decreased and calculation of kinetic rates was possible (see Table 2) but they were still generally lower than rates calculated from the IC-IPAD data (see Figure 5b). This confirms similar results observed with salt induced acid degradation of sucrose (5,6,7). Pol measurements were measured at 589 and 880nm wavelengths, but the 880nm values gave no significant information beyond that of the 589nm values.

Effect of Temperature

Temperatures (70 - 100°C) simulating commonly found unit process temperatures in sugar manufacture and refining, were studied on 65 Brix sucrose solutions under constant pH 8.3 conditions. pH 8.3 is a commonly encountered pH in the sugar industry and sucrose is stable (9) at this pH (also see Table 2). For each reaction temperature studied, the pH electrode was calibrated at that temperature using temperature extrapolated buffer pH values. A plot of color formation after 8 h against reaction temperature is shown in Figure 6, and indicates very little color formed between 70~85°C; moreover, no visually observed color was formed over 8h in the 70°C reaction. This indicates the strong kinetic influence of reaction temperature, even at such high sucrose concentrations. It was interesting to note that there was a direct linear relationship between color formation and titrant added (see Figure 7), whereas a non-linear relationship was observed in the constant pH effect reactions (see previous sections).

Effect of pH Electrode Calibration Parameters.

Effect of electrode calibration parameters was also studied on constant pH reactions. In sugar refinery operations pH electrodes are, unfortunately, sometimes calibrated with standard dilute aqueous buffers at room temperature, and then placed in high Brix sugar solutions at elevated temperature. However, equivalence does not likely exist (3). For all the constant pH reactions described in the previous sections, the electrode was calibrated using dilute standard pH buffers of 7 and 10, at the reaction temperature of 100°C. Moreover, as the buffer pH values are temperature dependent, the temperature extrapolated buffer pH values were used in the calibration.

Effect of pH electrode calibration parameters was further studied on 65 Brix sucrose solutions at constant pH 8.3. A reaction (denoted Δ) was undertaken at 100 °C, but the pH 7 and 10 buffers were calibrated at room temperature rather than at 100 °C. The electrode zero point was pH=7.07 and the calibration line slope =0.968. Titrant

(3M NaOH) added over 8 h was 2.154 mL, compared to 2.102 mL for the same experiment (denoted B) except the electrode was calibrated at 100°C using temperature extrapolated pH buffer values. Only slightly less color ($ICU_{420}=1317.8$) was formed in reaction A than B ($ICU_{420}=1400$) after 8h. A further reaction (C) was conducted with the same reaction parameters as in A and B, but this time the electrode was calibrated at 100 °C with buffers at pH 7 and 10, i.e., buffer values were not temperature extrapolated at 100 °C. The electrode zero point was pH=7.52 and calibration slope=0.815. In C, considerably more titrant was added over 8 h (9.15 mL) and a dark golden yellow formed ($ICU_{420}=5142.8$) after 8 h. Therefore, the electrode measured lower pH values when the calibration buffers were not reaction temperature extrapolated. These results confirm the need for correct calibration of electrodes at the reaction or unit process temperature, otherwise sucrose loss and formation of degradation products can be adversely affected and process control limited.

Organic Acid Degradation Product Formations.

The most accurate determination of sucrose loss in sugar manufacture and refining would be to analyse for a stable degradation product, i.e., a marker compound. An organic acid degradation product may be a viable marker and, consequently, organic acids formed in the various constant pH reactions were investigated using ion chromatography (IC). IC with conductivity detection is a sensitive and rapid technique for measuring organic acid anions. It has previously been reported (10) to be a useful technique to measure the anion composition of factory juices as well as white sugar.

Organic acids (<C6) formed in selected constant pH reactions are shown in Figure 8. The chloride and nitrate peaks were present in the ultrapure dilution water. As expected (3) marked increases in acetic, lactic and formic acid peaks were observed with increased pH. However, even with gradient modifications, acetic and lactic acids were difficult to quantitatively separate properly, compared to the isolated and sharp peak of formic acid. Therefore, using this IC-conductivity technique, formic acid presently offers most potential as a marker compound.

“Fingerprint” Oligosaccharide Degradation Products.

Oligosaccharides are formed in the breakdown of sucrose and monosaccharides, under acid and alkaline conditions, and have high potential as possible stable marker

compounds. An IC-IPAD method, using a strong NaOAc gradient, was developed to separate oligosaccharides (up to 12 degrees of polymerization) in concentrated sugar solutions on the DionexTM Carbo Pac PA-1 anion exchange column (see EXPERIMENTAL section). NaOAc is more strongly eluting than NaOH and unlike a hydroxide gradient there is little change in pH across an acetate gradient; this is important because the PAD electrode is sensitive to pH change. Figure 9 illustrates "fingerprint" oligosaccharide IC-IPAD chromatograms of selected sucrose degraded samples reacted for 8h under various constant pH conditions. It can be seen that one of the very useful properties of the CarboPac PA-1 columns is that they can tolerate high overload levels of sucrose and still detect other sugars, such as oligosaccharides. Furthermore, extensive column washings between runs is not required.

Maltooligosaccharides and other oligosaccharide standards were used to assign degrees of polymerization across the chromatogram run profile. From Figure 9 it was observed that under alkaline conditions a marked kinetic peak was visible after the sucrose peak (denoted C). The large increase in this peak size from pH 10.1 to 10.45 reflects the large increase in color formed and titrant added. Under acid pH 5.45 conditions, two predominant peaks (A and B) were visible, and A was tentatively identified as 1-kestose. Kestoses are formed from the reaction of a fructose carbocation formed in the initial acid degradation of sucrose, with another saccharide mainly sucrose (11); kestoses are characteristic of cane sugar (12). Further identification of the oligosaccharide peaks shown in Figure 9 is currently being undertaken with the use of a fraction collector to obtain pure peak samples, which will be subsequently analysed using GC-MS and NMR techniques.

SUMMARY AND CONCLUSIONS

- * color formation is least between pHs ~4.4-7.0
- * total organic acid formation is concomitant with color formation
- * minimum sucrose degradation, under constant pH conditions at 100°C and 65 Brix solutions, is between pHs~6.45-8.5
- * under alkaline degradation conditions, polarimetry is not viable for measuring kinetic degradation due to the formation of compounds with a positive pol
- * the importance of careful pH electrode calibration and control is clear

- * formic acid has the most potential as an organic acid marker if IC-conductivity detection is used to separate the complex mixture of acid degradation products
- * IC-IPAD, with a NaOAc gradient in NaOH, is a viable method for separating and monitoring "fingerprint" oligosaccharides produced under acid and alkaline degradation of sucrose.

This work is part of an ongoing USDA project. One of the major aims of this project is to identify stable marker compound(s) in different unit processes in the sugar industry to accurately and easily determine actual chemical sucrose loss. Identification of degradation products formed in the model systems discussed in this paper is currently being undertaken and are being compared with products found in industrial samples.

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Table 1. Auto-titrator parameters for constant pH reactions.

Constant reaction pH	NaOH titrant (M)	Minimum dosing rate ($\mu\text{l}/\text{min}$)
4.4	0.05	6
5.45	0.1	2
6.45	0.2	2
7.5	0.6	6
8.3	3	2
8.5	3	2
9.25	3	2
10.1	3	25
10.45	3	200

Table 2. Sucrose degradation under constant pH conditions: first-order kinetic constants.

First-order kinetic constants at 100°C

Constant reaction pH	IC-IPAD		Polarmetry	
	$-k_1$ $\text{sec}^{-1} \times 10^{-6}$	std error $\text{sec}^{-1} \times 10^{-6}$	$-k_1$ $\text{sec}^{-1} \times 10^{-6}$	std error $\text{sec}^{-1} \times 10^{-6}$
4.4	9.8	2.7	12.0	0.38
5.45	9.9	2.8	1.4	0.23
6.45	2.1	0.64	n/a ^a	n/a
8.3	2.3	0.42	n/a	n/a
8.5	2.6	0.7	n/a	n/a
9.25	5.2	0.6	n/a	n/a
10.1	7.4	0.1	n/a	n/a
10.45	9.3	1.7	2.2	0.57

n/a=not applicable (see text)

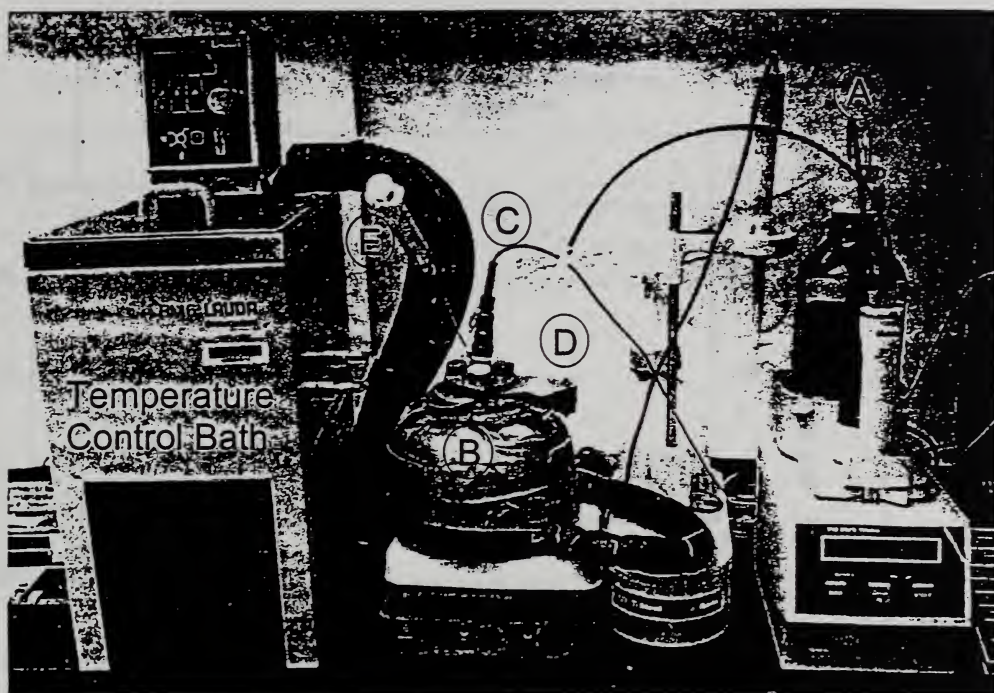


Figure 1. The constant pH auto-titration system. A=computerized auto-titrator, B=lagged reaction vessel, C=pH electrode, D=dosing tube, E=sample syringe.

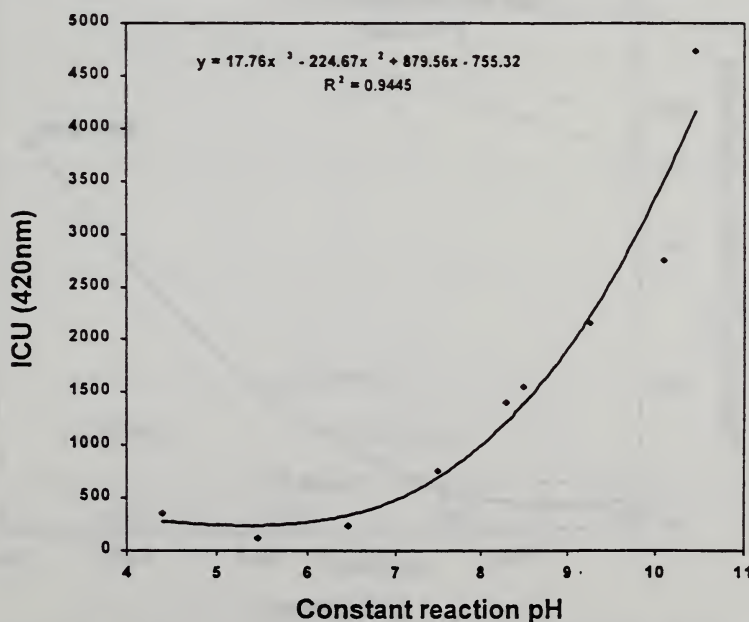


Figure 2. Effect of constant reaction pH on color formation after 8h sucrose (65 Brix; 100C) degradation.

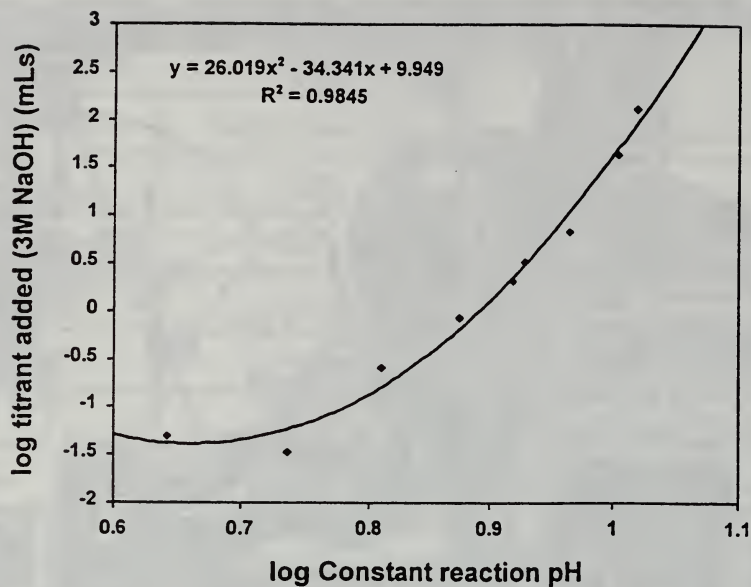


Figure 3. Effect of constant reaction pH on titrant added after 8h sucrose (65 Brix; 100C) degradation.

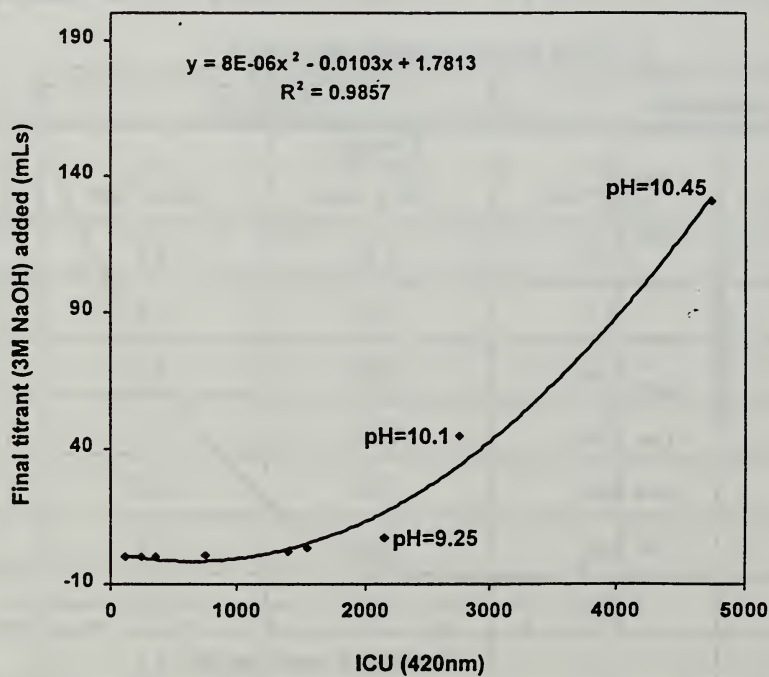


Figure 4. Effect of titrant added after 8h on color formation from sucrose degradation (65 Brix; 100C) under constant pH conditions.

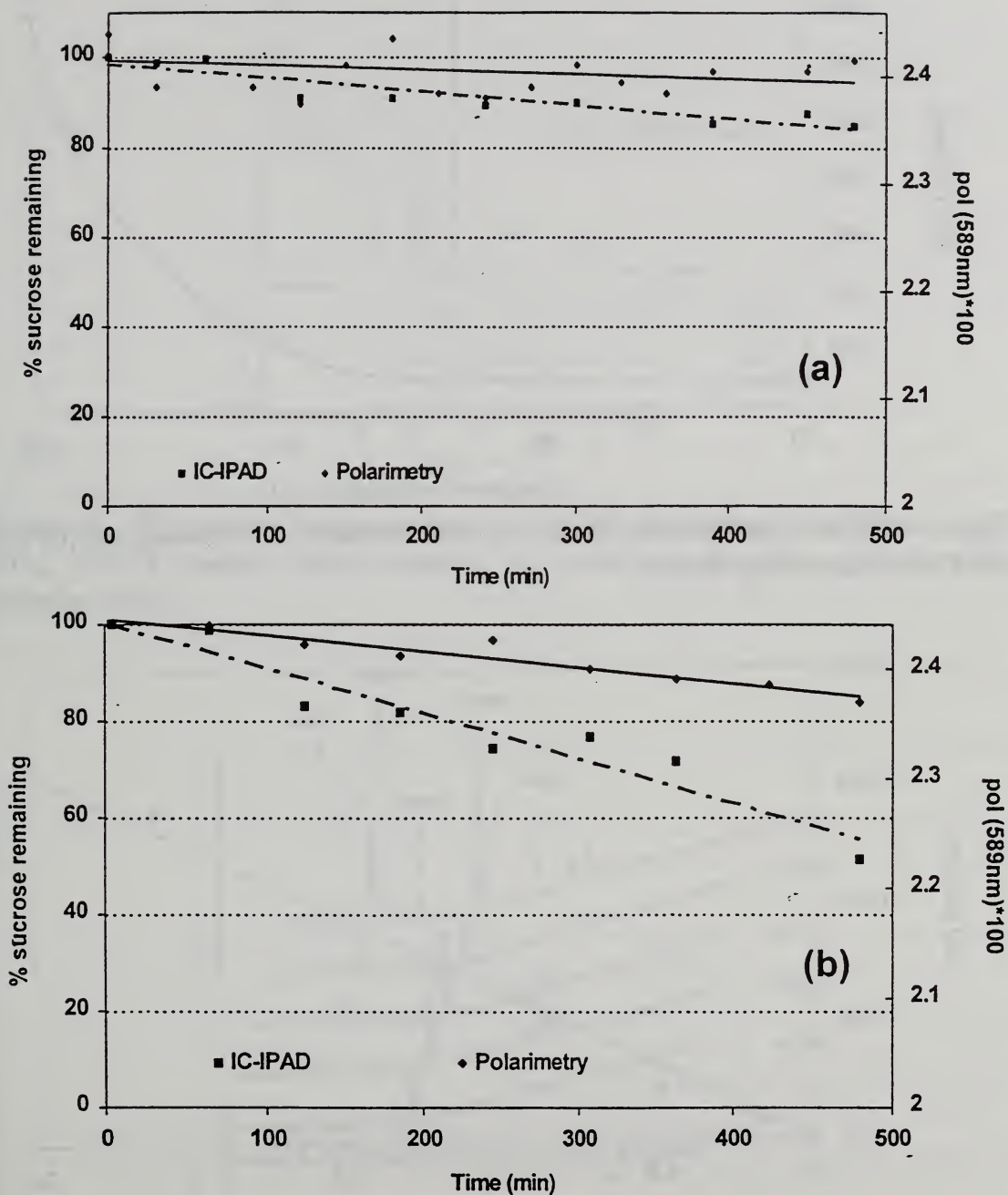


Figure 5. Use of IC-IPAD versus polarimetry to monitor sucrose (65 Brix; 100C) degradation under constant pH (a) 9.25 (b) 5.45 conditions.

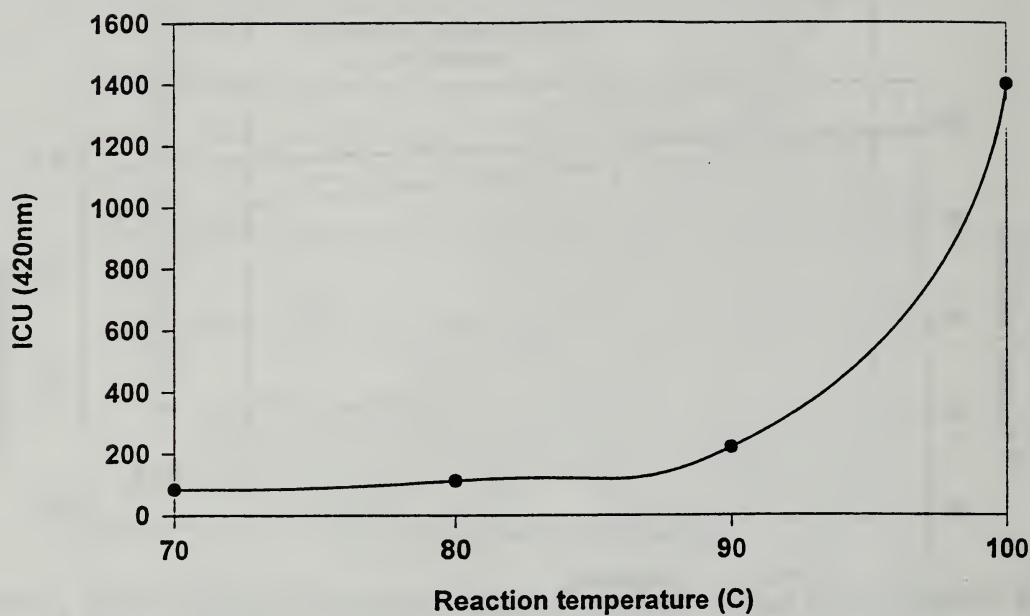


Figure 6. Effect of reaction temperature on color formation after 8h sucrose (65 Brix; pH 8.3 constant) degradation.

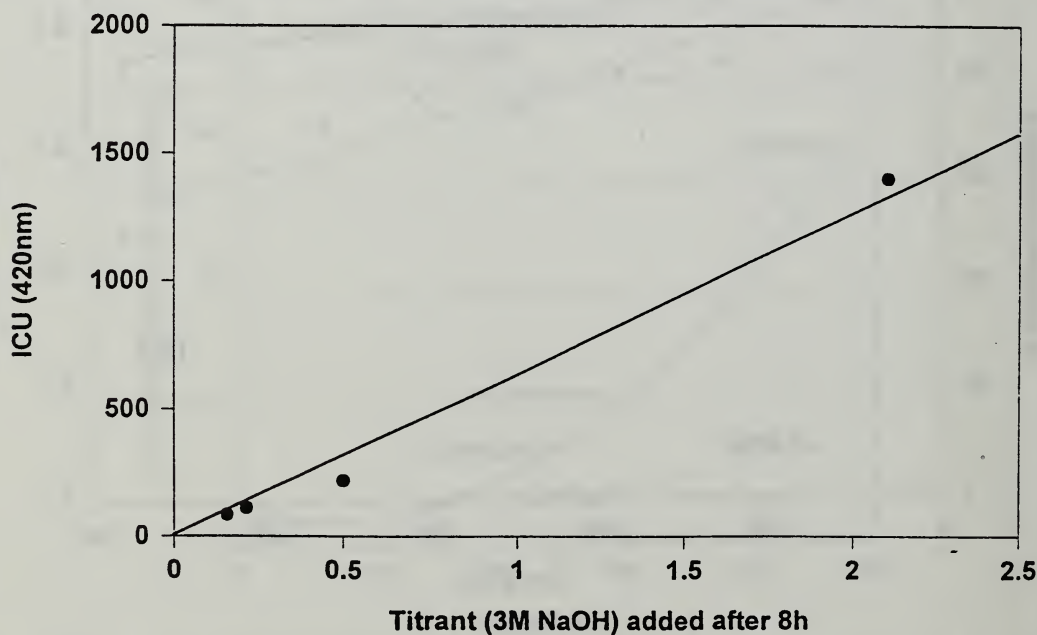


Figure 7. Effect of titrant added after 8h on color formation in sucrose (65 Brix; pH 8.3 constant) reactions undertaken at temperatures between 70 - 100C.

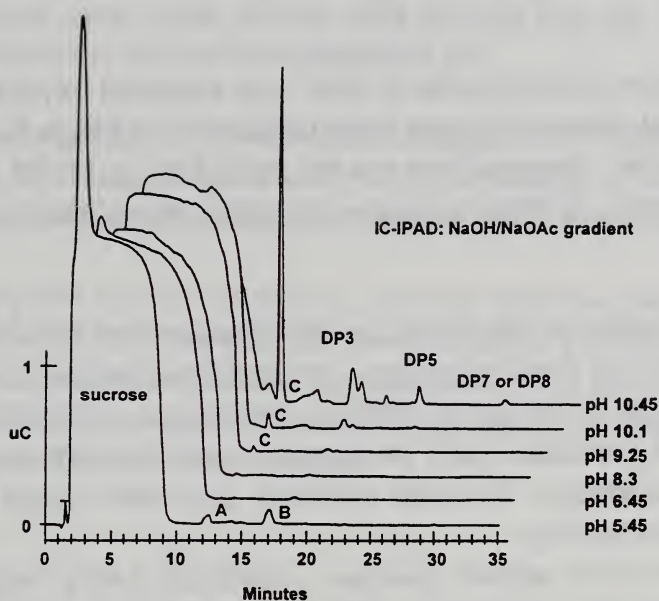


Figure 8. "Fingerprint" oligosaccharide IC-IPAD chromatograms of sucrose (65 Brix; 100C) reacted under constant pH conditions for 8h. DP=degree of polymerization.

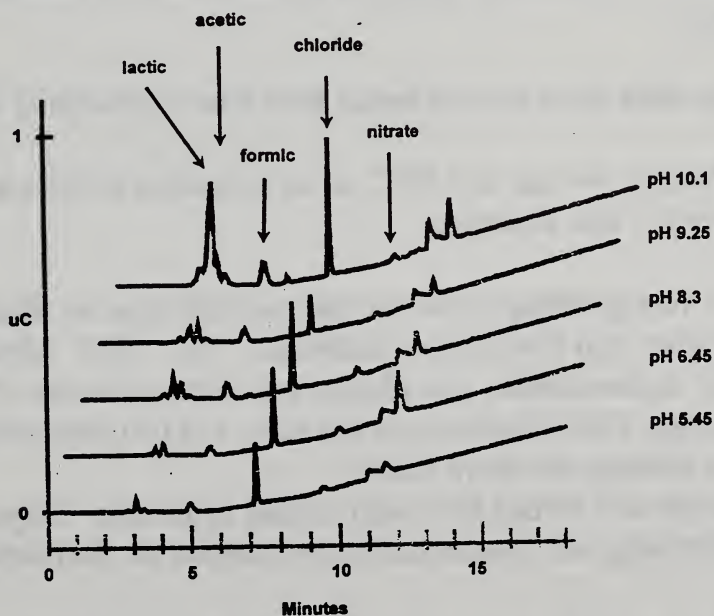


Figure 9. Organic acid IC-conductivity chromatograms of sucrose (65 Brix; 100C) reacted under constant pH conditions for 8h.

DISCUSSION

Question: From an industrial point of view: you excluded oxygen in your reactions. What effects might dissolved oxygen have, particularly on free radical chain reactions?

Another question: temperatures can run up as high as 140°C, in evaporator first bodies for example. Is there a chance you might extend the study to these higher temperatures?

Eggleston: The oxidation, obviously, can not be controlled in factories. That is why we did the reactions under nitrogen - to establish constant conditions and keep parameters constant. I can not comment on implications of dissolved oxygen, or on what degradation products would be formed - certainly there would be more effect on free radical mechanisms. For some processes, dissolved oxygen in factory streams is very undesirable anyway.

In answer to your second question: obviously 140°C would be difficult to simulate in this laboratory apparatus. We have, in another project, used Differential Scanning Calorimetry (DSC), to monitor sucrose degradation from 50°C to 500°C. That technique, or using an autoclave, could be used to look at behavior at 140°C, but those high temperatures occur only when there is occasional localized overheating, as in the evaporator. Most unit processes are not running anywhere near that temperature.

Comment: We think there are real losses from these overheating areas.

Eggleston: Perhaps we can use DSC or an autoclave technique to look at this problem and monitor that situation.

Comment: It is very gratifying to see that the research done on alkaline degradation of sucrose ten years ago (Reference - deBruijn, J. M. 1986. Monosaccharides in alkaline medium: isomerization, degradation and oligomerization, PhD thesis, Delft Univ. of Technology, The Netherlands) is still alive, and that new techniques are now being applied to develop this work further.

You maintain pH at a certain level with sodium hydroxide. Do you think there is any effect of increasing salt content during the reaction on the reaction rate?

Eggleston: Yes, we did a study using chlorides of various salts (Eggleston, et al. 1996. Effects of salts on the initial thermal degradation of concentrated aqueous solutions of sucrose. J. Carbohydr. Chem. 15:81-94) to look at effect of cation type

and concentration on reaction rate. There was some effect, but the least effect was with sodium ion - less than with calcium ion. We keep as many conditions constant as possible, to relate response to change in any one parameter.

We have also done model studies with calcium ion and CO_2 . That work, simulating carbonatation, has not been published yet.

Question: On the mechanism of your alkaline degradation of sucrose: in one slide I noted you see formation of lactic acid. So, you must have fructose or glucose present at these alkaline conditions to allow formation of lactic acid.

Eggleston: Compared to acid conditions, there are very low levels of fructose and glucose. In the old literature, the first sucrose breakdown products were thought to be glucose and fructose, even in alkaline conditions. But current thought is that sucrose first goes through a nucleophilic substitution reaction in degradation - but not through glucose and fructose intermediates.

RECENT PROGRESS IN SUGAR COLORANTS

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ABSTRACT

The study of color and color precursors in cane and beet sugar has been an important research area at SPRI for many years. Previous studies on isolating colorant precursors had relied on various liquid-liquid extraction procedures, methods that require large amounts of sample and solvent and that are time consuming. In this paper, several procedures are described for isolating sugar colorants that rely on micro-extraction methods, some using as little as 3 g of sample and only a few ml of solvent. Four methods were compared: Micro-extraction cartridges containing either anion exchange resin or C-18, Empore-SDB membranes, XAD-16 macroporous resin, methanol/ethyl acetate extraction. Colorants and colorant precursors in raw sugars from various sources were examined by gas chromatography and mass spectrometry. The presence of fructose anhydrides and a dimer of 5-hydroxymethylfurfural (HMF) is reported in both cane and beet colorant extracts.

INTRODUCTION

The understanding and control of color development in sugar processing is one of the constant challenges in the sugar industry. As early as 1916, researchers in Louisiana were already recognizing the importance of polyphenol-iron complexes in cane juice as a source of coloring matter (Schneller, 1916). In 1947, Zerban made the statement, "The darkening of sugar products during processing and storage at elevated temperatures is one of the oldest problems of sugar manufacture and makes itself felt in practically all other industries that use these products" (Zerban, 1947). In 1967, Gross made the statement, "The challenges of the highly complex question of the nature of sugar colour has been with the industry for a very long time" (Gross, 1967). In 1971, Smith and Gregory made the statement, "Sugar colour with its associated impurities and precursors is a complex mixture of diverse composition and is difficult to describe in practical terms" (Smith and Gregory, 1971).

It is obvious that research on sugar color has been going on for a very long time. It is equally true that it is a complex subject with many ramifications. The knowledge

gained has been incremental and has come from numerous sugar producing areas, encompassing all aspects of the process, starting with the coloring matter that is introduced by the cane or beet plant and continuing through reactions that occur in the mill and the refinery and on into storage.

There are various ways to classify sugar colorant. Among the general categories of sugar colorant are included those that originate in the plant and those that originate in the process (Clarke, et al., 1984).

Colorants that originate from the sugarcane plant include true pigments such as chlorophyll, anthocyanins, and flavonoids. Chlorophyll, being water insoluble, is easily removed, producing only some minor brown degradation products that are found in raw sugar (Roberts, 1980, internal SPRI report). The red pigments, the anthocyanins, are also a minor component which are largely removed or destroyed in clarification, but a small amount is found in polymeric colorant in raw sugar (Godshall and Grimm, 1994). Flavonoids include a wide range of compounds that are in the yellow range, and which may react further to form highly colored polymeric colorant.

An important group of plant-derived colorants and colorant precursors includes the many polyphenolic compounds, the benzoic and cinnamic acid derivatives, which range from colorless to yellow, darkening with alkaline pH and able to produce highly pigmented iron complexes. The phenolics are reactive and easily oxidized. Color formation involving enzymatic polymerization of polyphenolics in juice is estimated to be a major source of coloring material in both cane and beet processing (Gross and Coombs, 1976a, 1976b; Paton, 1992b).

Amino acids and reducing sugars are not colorants, but their presence and the reactions they undergo contribute to color formation in processing; they fall into the category of colorant precursors.

Color is also formed during processing, and the general categories of process-derived color, mostly polymeric and of a high molecular weight, include:

- Caramels -- thermal degradation products of sucrose

- Melanoidins -- Maillard reaction products of α -amino acids and reducing sugars

- Alkaline degradation products of fructose -- similar to caramels

Melanin -- reaction products of amino acids with phenolics; also, the very dark enzymatic oxidation products of phenolics (more common in beet colorant than cane).

Sugar colorant may also be classified on the basis of its molecular weight distribution, its ionic charge, and its pH sensitivity.

EXPERIMENTAL

Raw sugars were obtained from sponsoring companies. The geographic origin, color and date of production of the six sugars examined are listed below.

<u>Origin</u>	<u>Produced</u>	<u>pH</u>	<u>Color</u>
Fiji	9/95	5.91	2227
Louisiana	11/95	6.55	2812
Guatemala	8/94	5.97	2685
Dominican Republic	6/94	6.10	2978
Mozambique	9/94	5.52	6667
Brazil	11/94	4.65	11,972

Solid Phase Extraction. Four solid phase extraction media were examined:

- (1) Maxi-Clean® (Alltech) solid phase extraction cartridges containing a reverse phase packing (C18);
- (2) Maxi-Clean® (Alltech) solid phase extraction cartridges containing a strong anion exchange packing (SAX);
- (3) Empore®-SDB membranes embedded with styrenedivinylbenzene; and
- (4) XAD-16 macroporous resin.

The hydrophilic C18 packing was expected to extract moderately polar to non-polar compounds from a polar matrix, while the SAX packing was expected to remove compounds of an acidic nature from an aqueous solution. The Maxi-Clean® cartridge bed size was 600 mg, which is rated as being able to remove up to 6 mg of material. The completeness of extraction, however, depends on how strongly a compound is retained and the presence of other components in the mixture. Both the Empore®-

SDB membranes and XAD-16 resin are composed of a similar aromatic matrix, which is expected to have an affinity for aromatic, hydrophilic compounds.

Extraction procedure for SAX cartridges: 3 g sugar was dissolved in 20 ml water; the solution was filtered on a bed of analytical filter aid and passed through a SAX cartridge. It was not necessary to condition the SAX cartridges. The solution was passed sequentially through two cartridges. If a significant amount of color remained in the solution after passage through 2 cartridges, a third cartridge was used. (Developmental work had shown that 94-95% of all low molecular weight material was extracted in 2 cartridges, regardless of the amount of color remaining.) Each cartridge was then washed of sugar and salts with 30 ml water. The acids were eluted from each cartridge by 3 ml 3N formic acid followed by 3 ml water. The pooled eluates were evaporated to dryness. The dried extracts were redissolved in pyridine and an aliquot taken for derivatization as the trimethylsilyl (TMS) derivative with Tri-Sil Concentrate® (product of Pierce Chemical Company), using trehalose as the internal standard.

Extraction procedure for C18 cartridges: 5 g raw sugar was dissolved in 20 ml water and prefiltered on analytical filter aid and passed sequentially through two methanol/water conditioned C18 cartridges. Each cartridge was washed with 20 ml water and adsorbed compounds eluted with 3 ml methanol followed by 2 ml ethyl acetate. Pooled eluates were evaporated to dryness and the extracts prepared as described above for GC/MS. The effect of acidifying the sugar solution prior to extraction was also examined.

Extraction procedure for Empore-SDB membrane: 25 g raw sugar was dissolved in 50 ml water, filtered on a bed of analytical filter aid, and passed through a conditioned membrane. The membrane was conditioned with methanol followed by water and kept wet. Sugar was washed off the membrane with about 100 ml water. Color was eluted with 3 ml methanol followed by 3 ml 50% aqueous methanol. The extract was evaporated to dryness and prepared for GC/MS in the manner described above.

Extraction procedure for XAD-16 macroporous resin. Three standard beet liquors were examined using this procedure. 100 g liquor was diluted with 200 ml water and passed through a column (5 x 12 cm) filled with conditioned XAD-16. The XAD-16 was conditioned by rinsing with 4% NaOH and 5% HCl followed by water until neutral. The sugar solution was passed slowly through the column and sugar and other unretained material washed off with one liter of water. The retained colorant

was eluted with 50 ml ethanol followed by 50 ml methanol. The alcohols were evaporated off and the solution, in a small volumn of water, was freeze dried. The dried material was further extracted by stirring with methylene chloride overnight. The solvent was filtered on a sintered glass funnel, evaporated to dryness, and the sample prepared for GC/MS.

Solvent Extraction

Liquid/liquid extraction. 25 g raw sugar was dissolved in 100 ml water and the pH adjusted to 2.5 or not adjusted, as required. The solution was extracted with three 50-ml aliquots of ethyl acetate. The extract was dried over anhydrous sodium sulfate overnight, filtered, and evaporated to dryness, and the sample prepared for GC/MS.

Methanol/ethyl acetate extraction. The procedure used was a modification of the procedure described earlier (Larrahondo, et al., 1995; Giraldo, 1995). 25 g raw sugar was stirred with 100 ml methanol for 4 hours and then left to soak overnight. The methanolic extract was filtered on a sintered glass filter and evaporated to dryness. The dried residue, which contained a lot of sucrose was redissolved in 10 ml water, the pH adjusted to 2.5, and extracted with three 20-ml aliquots of ethyl acetate. The ethyl acetate extract was prepared for GC/MS in the manner described above for liquid/liquid extraction.

GC/MS

Separation and identification was accomplished using a Hewlett Packard Series II Model 5890A gas chromatograph coupled with a Hewlett Packard 5972A mass selective detector (MSD). The MSD was equipped with a hyperbolic quadrupole mass filter and an electron impact ion source. The column used was 30m x 0.25cm with 0.25 μ m film thickness, 5% phenyl methyl silicone phase. Various temperature programming regimes were used, depending on the nature of the compounds being studied. Retention times reported in the tables in the appendix were obtained using an initial temperature of 100C; hold for 4 min; increase 4C/min to 250C and hold for 10 min.

Compounds were identified on the basis of their mass fragmentation patterns and retention times. A commercial mass spectral library from Wiley was used for comparison and identification of spectra, along with a SPRI library of standards.

RESULTS AND DISCUSSION

The quantitative results of the various extractions are presented in the appendix. Table A-1 compares the compounds extracted from six raw sugars using the acidified methanol/ethyl acetate procedure. Table A-2 shows the results of different extraction procedures on the extractables in a Fijian raw sugar. Table A-3 shows the effect of heat on the colorants in the Fiji raw sugar.

Extraction by Empore-SDB membranes resulted in the removal of most of the sugar color but recovery of only about half of the retained color. There was always a lot of sucrose remaining in the membranes, in spite of extensive washing. The Empore membranes did not extract small molecular weight colorants, other than a small amount of beta-sitosterol. The results indicate that these membranes are better suited to examination of high molecular weight colorant, and are not further discussed in this paper.

XAD-16 macroporous resin was used to examine beet evaporator syrup and is discussed in a separate section below.

What is a sugar colorant? The definition of sugar colorant can be extremely broad, with almost any component in a raw sugar that is not sucrose, reducing sugar, or inorganic salt falling into one or another category of colorant. In many instances, the identified compounds themselves are not colored but may have the potential of entering into color-forming reactions or to act as catalysts for color-forming reactions. The extracts discussed in this study yielded in excess of 100 individual compounds, including carboxylic acids, polyhydroxyphenolic acids, lactones, alcohols, lipids, furans, glycosides, amino acids, and at least one inorganic acid, phosphoric.

Most of the compounds discussed in this paper are not strictly colorants. They are either not colored or have light shades of yellow. However, because of their nature and abundance, they constitute a source from which color reactions can occur. The highly colored components of sugar colorant (dark brown, golden and reddish) have molecular weights ranging from 1000 to more than one million daltons. These high molecular weight colorants cannot be studied by our GC/MS system, which is limited to molecular weights below approximately 1000 daltons.

At least 60 of the extracted compounds were tentatively or definitely identified, or partially characterized, by the mass fragmentation patterns of their trimethylsilyl (TMS) derivatives.

Comparison of Colorants in Six Raw Sugars

The colorants in six raw sugars were compared using the acidified methanol/ethyl acetate extraction procedure. Results are presented in Table A-1 of the Appendix. As the discussion in the next section shows, this method, while putatively a surface-extracting procedure, gave the widest range of components in the highest over-all yields, and thus was chosen as the method used to compare raw sugars.

The first thing to note is the general over-all similarity among the sugars. Although they represent a diverse range of origins, many of the same compounds were found in each one, the main differences being those of quantity. The Guatemala, Fiji, Dominican Republic and Louisiana raws had low colors, in the range of 2000-3000 ICU. The Brazil raw had very high color, low pH and high reducing sugars and had been difficult to decolorize. The Mozambique raw had a moderately high color but had not been particularly difficult to refine.

Compounds which were found to be in higher concentration in the more highly colored Brazil and Mozambique raws included the unknown (m/z 73, 147, 205) at 3.85 min, 3-hydroxypropanoic acid (4.22 min), methyl-hydroxy-pentanoic acid (5.96 min), and succinic acid. The Brazil raw also had a higher concentration of 5-hydroxymethyl-2-furancarboxylic acid, a sugar degradation product. Aconitic acid was low in the Brazil raw, and could have been degraded; data discussed below shows the loss of aconitic acid in the presence of heat, with subsequent color formation. However, the concentration of aconitic acid in cane juice is a function of cane maturity (it is higher in immature cane), and a low concentration does not necessarily indicate its involvement in color formation. Aconitic acid was also low in the D.R. sugar, which did not have a color problem.

The Louisiana raw was generally higher in the phenolic acids, especially ferulic, caffeic and chlorogenic acids. It was also higher in some of the plant metabolic acids, such as aconitic, malic, fumaric, and 5-oxo-proline.

The Guatemala, D.R. and Louisiana raws were characterized by measurable amounts of a hexanedioic acid ester (the nature of the ester moiety was not determined). An

earlier extract of a very low color Louisiana raw sugar produced in 1991 (1306 ICU) had also shown a prominent hexanedioic acid ester peak. The Fiji and Mozambique raw had measurable concentrations of the triphenyl ester of phosphoric acid. The Brazil raw had 0.11 ppm of each of these esters.

Peaks such as the hexanedioic acid ester or the triphenyl ester of phosphoric acid may be characteristic of certain locations, but more study would have to be done on this.

Comparing Extraction Procedures

Five extraction procedures are compared in Table A-2 (Appendix). The modified methanol/ethyl acetate method (Larrahondo, et al., 1995; Giraldo, 1995) may be considered a surface extraction procedure -- that is, the procedure is more likely to strip the colorants out of the first few layers of the sucrose crystal than accessing the entire crystal. However, since the method calls for stirring the sugar with a magnetic stirring bar for four hours, the sugar crystals are broken into much smaller particles by the procedure. The breaking of the crystals makes more of the in-crystal colorant accessible to the methanol. At the end of the procedure it is noted that at least two-thirds of the color has gone into the methanolic layer.

The results of the surface extraction methods are shown in the first two results columns of Table A-2, one acidified and the other not acidified. The effect of including an acidification step in the procedure is especially marked in the extraction of aconitic acid, where 46 ppm was extracted from the acidified solution but less than 1 ppm was extracted from the same sugar when the solution was not acidified. This effect was confirmed by repeating the extraction of the Fiji raw sugar as well as two other sugars. Other carboxylic acids, such as lactic, malic, succinic, citraconic, etc., showed similar trends. Neutral components (lactic acid lactate, lactones) and less acidic components (ferulic acid, fatty acids) were extracted to about the same extent by both methods.

The results of the acidified ethyl acetate liquid/liquid extraction (third results column of Table A-2) can be used to interpret how much additional material was inside the crystals. The results showed no new compounds were found exclusively inside the crystal. Several compounds were present in higher concentrations than in the acidified methanol extract, indicating that they were partially occluded in the crystal. Among these were, lactic acid, aconitic acid, the unknown (m/z 145) at 7.35 min, citraconic acid, the fructose degradation product at 10.38 min (FDP-1), 5-

hydroxymethyl-2-furancarboxylic acid, resorcinol, ferulic acid and p-hydroxybenzoic acid. The difference in lactic and aconitic acids by the two procedures, however, was not very great. It should be noted that the compounds found inside the crystal also have a tendency to form more color or to be involved in degradation reactions.

While many constituents were present in approximately the same concentration as in the surface procedure, a few others were present in considerably lower concentrations; for example, 2-furancarboxylic acid, malonic acid, glyceric acid, and erythronic acid. A possible explanation for this is that the concentration of sugar relative to the extracted material was quite different, facilitating better extraction from the surface procedure than the liquid/liquid procedure. In an earlier study, the concentration of sugar was a factor in the efficiency of extraction (Godshall, 1975).

The last two columns of Table A-2 compare two solid phases in microcartridges, a strong anion exchange resin (SAX) and a hydrophobic silica-based reverse phase packing (C18). These extractions also represent the colorant in the entire crystal. The SAX cartridge showed a high affinity for most of the carboxylic acids, as well as the fatty acids, with the notable exceptions of malonic, fumaric and aconitic acids. It had little affinity for any of the phenolic acids nor for the neutral compounds. The yield of quinic acid (23.40 ppm) greatly exceeded that of the other procedures.

Table 1 compares the SAX extract of the Mozambique and Fiji raws to the acidified methanol/ethyl acetate surface procedure for selected compounds. The compounds that are extracted in significantly higher quantity by SAX include malic, aspartic, quinic, palmitic, stearic, citric, and gluconic acids as well as glucono-delta-lactone and 5-oxo-proline.

The large concentration of glucono-delta-lactone and gluconic acid obtained by the SAX extraction and the absence of these in the other extraction procedures was noted. We wondered if this was an artefact of the extraction procedure, possibly being formed by the action of formic acid on residual sugars in the extract, as the formic acid was being evaporated off. Several control experiments were conducted: In one, refined sugar was passed through SAX cartridges and worked up in the usual manner. Neither glucono-delta-lactone nor gluconic acid was observed. In a second control experiment, sugarcane indigenous polysaccharide (ISP) was treated with formic acid in a similar manner to the extracts, and then analyzed by GC/MS, also with negative results.

The C18 cartridge had very little affinity for carboxylic acids and minimal affinity for phenolic acids, but it did extract comparable amounts of palmitic and stearic acids. It also extracted two steroids, stigmasterol and sitosterol. The C18 procedure was repeated several times on this and other sugars with similar results each time. Acidifying the sugar solution prior to extraction enhanced extraction, and the acidified results are shown in Table 2-A. Of the five procedures listed, the C18 cartridge gave the least satisfactory results and provided no additional information to supplement the other methods.

The Effect of Heat on Raw Sugar Colorants

The results of heating the Fiji raw sugar for 4 hours at 120C are shown in Table A-3. Heating this sugar increased the color from 2201 to 8608 ICU, resulting in a redder hue than the yellow color of the untreated sugar. Several changes in the pattern of the extraction due to heat are noteworthy.

The two biggest changes were the large increase in FDP-1 (m/z 273) at 10.38 min, from 0.68 ppm to 37.17 ppm, and the loss of much of the aconitic acid, from 45.9 ppm to 10.6 ppm. FDP-1, discussed later in this paper, is also a major product of alkaline degradation of fructose, and a minor product in acid degradation of fructose. Other sugar degradation products that increased significantly were 2-furancarboxylic acid, 3-hydroxy-propanoic acid, glyceric acid, citraconic acid, FDP-2 (m/z 271), lactic acid lactate, 5-hydroxymethylfurancarboxylic acid, and the lactones at 11.71, 14.14 and 14.20 min.

Lactic, malic and erythronic acids remained unchanged.

Figure 1 compares a portion of the chromatogram of the heated raw with the unheated raw, showing the increase in FDP-1 and other constituents upon heating.

Cane Juice as the Origin of Many Colorants

Many of the compounds found in Table 1-A originate in the cane juice, and varietal differences are known to occur among flavonoids and other phenolics in sugarcane (Paton, 1992c; Smith and Paton, 1985). Phenolics also increase in the cane as it matures (Godshall and Legendre, 1988).

Cane juice was examined as the source of many of the colorant precursors in raw sugar. Fresh cane juice was freeze dried and approximately 100 mg of the dried residue dissolved in pyridine with 25-30 mg trehalose internal standard. Two hundred microliters of this solution was derivatized for quantitative determination by GC/MS. Juice from two varieties, both important commercial varieties in Louisiana, one with high juice color (CP 72-370) and one with low juice color (CP 70-321), were examined. The results are presented in Table 2. There is little difference between the two varieties in concentration of the major constituents measured in Table 2.

Because of the large amount of interfering sucrose present in the whole, freeze-dried cane juice, it was not possible to quantitate the less concentrated components. Therefore, approximately 8 g of the remaining freeze dried cane juice solids were stirred with ethyl acetate (as is, without redissolving the cane juice) and measured using trehalose as the internal standard. GC/MS confirmed the presence of many of the components listed in Table A-1. Found in the range of 0.5 to 10 ppm were: Succinic acid, fumaric acid, p-hydroxybenzoic acid, syringic acid, p-hydroxycinnamic acid, palmitic acid, stearic acid, and p-hydroxybenzaldehyde (not found in the raw sugars, and present only in variety CP 72-370). The samples were not examined for lactic acid, as the GC temperature program used was too high to include lactic acid.

Also present in concentrations ranging around 5-15 ppm were the tentatively identified phenolic glycosides and glycerol esters discussed below, with prominent mass fragments for individual peaks of m/z 267, 269, 297 and 327. This paper will not address the differences between the two sugarcane varieties except to point out that many of the organic acids and most of the phenolics in raw sugar originate in the cane juice and are carried over through the manufacturing process. Further quantitative work may show that the phenolic glycosides contribute to the higher color of CP 72-370.

Sugar degradation indicators such as glyceric acid, lactic acid lactate, FDP-1 and FDP-2 were not present in either variety.

Beet Sugar Colorant

Phenolic acids and phenolic amines are implicated in color formation of sugarbeet juice (Winstrom-Olson et al, 1979a, 1979b) as well as many flavonoids (Maurandi, 1988). Three standard beet liquors were extracted using XAD-16 with ethanol/methanol/methylene chloride as the extracting medium. One relatively low

color liquor (X) had produced a high color white sugar. The other two liquors (Y, Z) had produced normal white sugars. The extract of the X-liquor showed the presence of HMF and HMF dimer along with three fructose dianhydrides, several phenolic aldehydes and several acids. The extracts of Y and Z liquors lacked most of these compounds, but did have small amounts of the fatty acids and some of the phenolics. Also present in liquor X were several nitrogenous compounds, not identified at this time, with concentrations in the range of 0.2-0.5 ppm on solids. These were also present in liquors Y and Z.

Table 3 shows the quantitative results for compounds identified in liquor X. The low concentrations are noted. However, HMF, HMF dimer, and fructose anhydrides are strong indicators of acid fructose degradation and may explain why the white sugar produced from this syrup was high in color. If any of these compounds had carried over into the sugar, it would also probably have a tendency to increase in color with time.

Figure 2 presents a portion of the chromatogram of the extract of liquor X, showing the three identified difructose dianhydrides.

Discussion of Selected Groups of Compounds

This study has shown that many compounds are present in raw sugar, and the question arises, What is the significance of this information? Why should we be interested in the trace components of raw sugars? The presence of numerous compounds in raw sugar emphasizes the complexity of the challenge faced by the sugar refiner. There are many compounds to be removed, each with its own kinetics for removal in process, tendency to increase or decrease in process, with concomitant color formation, and affinity for inclusion in the sugar crystal.

The following sections briefly discuss several groups of compounds that are of particular interest in the study of sugar color.

Fructose Degradation Products. The degradation reactions of fructose in both acid and alkaline conditions have been studied extensively (Hodge, 1953; Isbell, et al., 1969; McWeeny, 1973; Newth, 1951; Shallenberger and Birch, 1975; Shallenberger and Mattick, 1983; many others). The absence of 5-hydroxymethyl-2-furfural (HMF), a major degradation product of fructose under acid conditions and also produced under alkaline conditions, in all the sugars studied and by all extraction

methods was noted. HMF was easily extracted into ethyl acetate from alkaline and acid-degraded fructose solutions prepared in the laboratory, and was identified in one of the standard beet liquors, so its absence was not an artefact of the extraction procedures used.

Three fructose degradation products (FDP's) were found in some of the sugars discussed in this paper. The structure of two of these is still under investigation and the third is interpreted as a dimer of HMF. These are discussed below.

(1) Mass 273 compound at 10.38 min. (FDP-1) This compound was present in all but the Mozambique raw sugar. The same compound was noted as a very minor peak in acid degraded fructose prepared in the laboratory and as a moderately large peak in alkaline degraded fructose. Its concentration in the Fiji raw increased more than 50-fold upon heating.

The mass fragmentation pattern of the TMS derivative is shown in Figure 3. One possible structure is partially hydrated 5-hydroxymethyl-2-furancarboxylic acid, $C_6H_8O_4$, which would have a TMS molecular weight of 288; the loss of a methyl group (15 amu) would give the prominent m/z 273 peak.

The relative mass abundances of the major fragments are given (The first number is the molecular weight of the mass fragment and the number in parentheses is the relative abundance as a percentage of the fragment relative to the most abundant fragment): 73 (100), 101 (12), 116 (6), 129 (9), 133 (7), 147 (29), 155 (22), 183 (23), 217 (2), 245 (5), 273 (51), 288 (4).

(2) Mass 271 compound at 11.10 min. (FDP-2) This compound was present in trace quantities in the Guatemala, Brazil, Fiji, and Dominican Republic raws and absent in the Mozambique and Louisiana raws. The same compound was noted as a minor peak in both acid and alkaline degraded fructose prepared in the laboratory. Its concentration increased in the heated Fiji raw from 0.03 ppm to 0.48 ppm.

The mass fragmentation pattern of the TMS derivative is shown in Figure 4. The TMS molecular weight of this compounds is probably 286; the loss of a methyl group (a common pattern in the MS of TMS derivatives) from the parent ion would account for the prominent m/z 271 fragment. The structure is of this compound is not known at this time, but it is speculated that it is derived from HMF.

The relative mass abundances of the major fragments were: 73 (19), 117 (1), 128 (3), 133 (4), 147 (4), 169 (2), 183 (1), 199 (7), 271 (100), 272 (23), 273 (11), 286 (0.4).

(3) HMF dimer. This compound was found in the extract of a standard beet liquor that had produced white sugar with high color. It was also noted as a major peak in the colorant extract of a Colombian raw sugar reported previously (Larrahondo, et al., 1995; Larrahondo, et al., 1996). It was a major peak in acid degraded fructose prepared in the laboratory but was negative in alkaline degraded fructose. It was not found in the 6 raw sugars examined in this study.

The mass fragmentation pattern of the TMS derivative is shown in Figure 5 along with a proposed structure. The TMS molecular weight of this compound is estimated to be 452.

The relative mass abundances of the major fragments were: 73 (71), 109 (14), 147 (6), 182 (4), 271 (100), 272 (23), 273 (10), 378 (3), 452 (0.3).

(4) Diffructose dianhydrides. In acidic medium, fructose can dehydrate to form a series of at least seven non-reducing dimeric dianhydrides (Hilton, 1963). These have the trivial names diheterolevulosan I, II, III, and IV, and diffructose anhydride I, II, and III, abbreviated as DHL and DFA, respectively. Alternate anomeric structures are also possible. Heat alone may also produce diffructose dianhydrides (DFDA). The presence of DFDA's in raw sugar or any other process sample would be another indicator of sucrose loss, in this case, due to thermal and/or acidic conditions.

In this study, extraction conditions were not optimized for the examination of DFDA, but they were noted in the XAD-16 extract of a beet sugar liquor and traces were noted in the methanolic extract of the Fiji heated sugar.

Other Sugar Degradation Products. Although fructose is generally recognized as being more reactive than glucose and participating more readily in degradation and color-forming reactions, glucose also undergoes oxidation and degradation in alkaline media. In the presence of air and calcium hydroxide, glucose, upon oxidation, yields carbon dioxide, formic acid, oxalic acid, arabonic acid, erythronic acid, glyceric acid, glycolic acid, and glucic acid (Gortner, 1938). At the turn of the century, Nef (1907, 1914) showed that in the presence of sodium hydroxide, glucose could react to yield an equilibrium mixture containing at least 93 different compounds.

In the present study, measureable amounts of erythronic acid (trihydroxybutyric acid) and glyceric acid (2,3-dihydroxypropionic acid) were found in the six raw sugars examined.

Saccharinic acids and saccharinic acid lactones. Saccharinic acids are formed by internal oxidation and reduction of aldomonosaccharides and are commonly formed in alkaline solution (Gortner, 1938; Sowden, 1957). Three forms are recognized, having the trivial names saccharinic acid, isosaccharinic acid, and metasaccharinic acid; they are isomeric with their starting sugars, and have a strong tendency to convert to the more stable lactone.

Saccharinic acids have been described in beet sugar processing (deBruijn, et al., 1987; Reinefeld, et al., 1979).

In the present study, several peaks in the raw sugar extracts appeared to be saccharinic acid lactones, based on their spectral matches with the commercial library. These included four lactones, tentatively identified as the following:

11.71 min:	2-deoxy-erythropentono-1,4-lactone
14.14 min:	xylonic acid gamma lactone
14.20 min:	2-C-methyl ribonic acid gamma lactone
17.09 min:	3-deoxy-arabino-hexonic acid gamma lactone

The lactones increased significantly in the heated Fiji raw compared to the unheated sample. Two other tentatively identified isomeric lactones were noted in the heated Fiji raw sugar. These are characterized by an ion at m/z 348 (Ponder and Richards, 1993), and increased about 5 times in concentration when the sugar was heated. The ion chromatograms for these compounds are compared in the heated and unheated sugar in Figure 6. The two peaks are tentatively identified as isomers of 3-deoxy-2-C-hydroxymethyl-D-erythro-pentonic acid gamma lactone.

Many compounds remain to be identified in the raw sugar extracts. Many of the unknown peaks have mass ions characteristic of hexoses and hexose degradation

products (m/z 147, 191, 204, 217), indicating that they may represent other closely related glucose and fructose dehydration, rearrangement and degradation products.

Phenolic Compounds

As discussed above, phenolics are ubiquitous constituents of both cane and beet plants and play a number of important roles in secondary metabolism. They are implicated in enzymatic formation of polymeric colorant and many are found in raw sugar.

Chlorogenic Acid and Other Quinic Acid Esters. Chlorogenic acid was first reported in sugarcane in 1971 (Farber and Carpenter, 1971; Gross and Coombs, 1971). Chlorogenic acid is the trivial name of 5'-caffeoylquinic acid. At least 7 quinic acid esters are found in plants (Trugo and Macrae, 1984), including esters of caffeic acid, ferulic acid, dicaffeic acid and p-coumaric acid. Paton (1992a) identified 3'-caffeoylquinic acid, 3'-p-coumaroylquinic acid and 4'-caffeoyl-quinic acid in cane juice in Australia, along with chlorogenic acid. Chlorogenic acid and related compounds are important color precursors in cane sugar processing.

Both chlorogenic acid and quinic acid were identified in all of the raw sugars examined (cf, Table A-1). The SAX extraction procedure showed that a large amount of quinic acid was present inside the crystal in at least two sugars (53 ppm in Mozambique and 23 ppm in Fiji, cf, Table 1), and it was present as the free acid at about 75-80 ppm on solids in two varieties of cane juice (cf, Table 2).

Quinic acid is characterized by a major ion at m/z 345 (46%); this ion is also present in a relative abundance of 27% in chlorogenic acid. In this study, the Fiji, Guatemala, Dominican Republic, and Louisiana raws exhibited 6 other peaks containing m/z 345 ranging in abundance from 12% to 28%, with retention times from 34 min to 43 min. These peaks may be other quinic acid esters.

Traces of six of these peaks were present in the high color Brazil and Mozambique raws, but their very low levels are in marked contrast to the other (low color) sugars. It is possible that these components have degraded to form color in the Brazil and Mozambique sugars. Figure 7 shows the seven m/z 345 peaks found in the Fiji raw sugar and contrasts that to the Brazil raw.

Phenolic Glycosides. MS indicated the presence of several phenolic glycosides. Palla (1982, 1983) reported the isolation and identification of phenolic glucosides in

cane molasses. The aglycones were a series of phenyl glycerols (phenyl propanetriols). We used his reported MS fragmentation patterns as guides to search for the presence of similar compounds in our extracts. Three sets of compounds were tentatively identified.

(1) Mass 297 compounds. The TMS derivative of 3-methoxy-4-hydroxy-phenyl-glycerol (*threo*-form) shows the major mass fragment to be m/z 297 (Palla, 1983). A search for this ion in the MeOH/EtAc extract of the Fiji raw sugar yielded 3 peaks in which m/z 297 was a major ion. The first peak had a retention time of 20.81 min (m/z 297 = 75%), and was present at a concentration of 0.36 ppm in the Fiji raw sugar. It was also present in trace quantities in the Guatemala, Brazil, Mozambique, and Dominican Republic raws. The Louisiana raw had a concentration of 0.91 ppm. The mass fragmentation pattern shows ions typical of glycerol (m/z 103, 117, 133). Its retention time suggests that this is the aglycone, with a TMS/MW of 502.

The other two peaks had retention times of 35.73 min (m/z 297 = 100%) and 38.03 min (m/z 297 = 94%). The retention times, as well as the presence of mass fragments characteristic of glucose (m/z 191, 204, 217) in both, suggest that these are monoglucosides.

(2) Mass 269 compound. The TMS derivative of 3-methoxy-4-hydroxy-phenyl-glycerol (*erythro*-form) shows a major mass fragment at m/z 269 (77%) (Palla, 1983). A search for this ion in the MeOH/EtAc extract of the Fiji raw sugar yielded one peak with a retention time of 21.42 min in which m/z 269 represented 44%. This is tentatively identified as the named compound. It was present in low quantities in all of the sugars, with the Louisiana raw having the highest amount, 0.70 ppm.

There were no compounds with higher retention times with a prominent m/z 269 fragment that could be interpreted as glycosides of this compound.

(3) Mass 327 compounds. The TMS derivative of 3,5-dimethoxy-4-hydroxy-phenyl-glycerol shows the major mass fragment to be m/z 327 (100%) (Palla, 1983). A search for this ion in the MeOH/EtAc extract of the Fiji raw sugar yielded 3 peaks in which m/z 327 was the major ion. The first peak had a retention time of 22.12 min. The mass fragmentation pattern shows ions typical of glycerol (m/z 103, 117, 133). Its retention time suggests that this is the aglycone, with a TMS/MW of 532.

A second peak with retention time of 22.47 had a prominent m/z 327 (32%) ion, but also prominent fragments at m/z 253 (33), 355 (39), and 370 (23), indicating that it is also a phenolic compound, but its structure has not been determined.

The other two peaks had retention times of 40.98 and 41.22 (m/z 327 = 100% in both). The retention times, as well as the presence of ions characteristic of glucose (m/z 191, 204, 217) in both, suggest that these are mono-glucosides.

Figure 8 shows the structures of the three phenyl glycerol aglycones discussed above.

(4) Mass 267 compounds. Two closely eluting and well-defined peaks with retention times of 24.85 and 25.16 min with major mass fragment of m/z 267 (100%) were observed. These compounds are closely related structurally to 4-hydroxy-phenyl (α -hydroxy) acetic acid (87% and 74% similarity, respectively), but have a higher molecular weight, indicating the presence of another substituent. Their structures were not further characterized at this time, except for the observation that these represent two more phenolic compounds found in all 6 raw sugars examined.

Miscellaneous Compounds

(1) Unknown (m/z 145), 7.35 min. An unidentified compound eluting at 7.35 min, with a major ion at m/z 145 (94%) was noted in all of the raw sugars, using the methanol/ethyl acetate procedure (surface), in a concentration range of 1-3 ppm. The different extraction procedures indicated that it was not acidic and was distributed throughout the crystal (cf, Table A-2). It appears to be stable to thermal degradation (cf, Table A-3). The presence of the m/z 73 ion indicates that it has at least one active hydrogen, either a hydroxyl or an amine. Its mass spectrum is shown in Figure 9.

(2) Hexanedioc acid ester, 25.95 min. This compound was discussed above. It was prominent (3-4 ppm) in the Guatemala, D.R. and Louisiana raws. An extract of another Louisiana raw from a different factory, produced in 1991 also presented this compound as a major peak. Hexane-dioic acid (adipic acid) is found in beet juice (Merck Index, 11th Edition, 1989), but its presence in cane juice has not been reported. The nature of the ester moiety was not determined.

(3) Phthalate, 27.78 min. The dibutyl ester of phthalic acid was found in all of the raw sugars and was extracted by all of the procedures examined. It is ubiquitous in cane

products, having been found in almost all process samples examined. It may result from storing samples in plastic bottles. The SAX cartridges were negative as a source of phthalate contamination.

(4) Glycerol, 6.85 min. Glycerol was found in the six raw sugars and also noted in the fresh cane juice samples. Glycerol is formed by fermentation of sugars and by alkaline saponification of fatty acid mono-, di-, and triglycerides. It may also be a normal cane constituent.

(5) Fatty acids. Four fatty acids were found in the sugars -- palmitic, oleic, stearic, and 9,12-octadecadienoic acid. These acids were also found as the free acids in both varieties of fresh cane juice examined in this study.

SUMMARY

This study has examined the constituents in six raw sugars of various origins, finding that each sugar has well over 100 peaks, representing a wide range of chemical types, including sugar degradation products, plant pigments, colorant precursors, and potential catalysts of further sugar breakdown (ie, organic acids). Many of the compounds that remain unidentified may be carbohydrate degradation products, based on the nature of their mass spectra. The sugars show quantitative differences in the amounts of constituents present.

Several extraction methods were examined, and each method, except for the C18 extraction cartridges, was able to provide useful additional information. The SAX cartridges are especially convenient and easy to use, and provide a better quantitative picture of the acidic compounds in the whole crystal than any other method. The methanol/ethyl acetate method provides information about constituents on the surface of the crystal. Liquid/liquid extraction is still a recommended procedure for the quantitative examination of the general range of sugar constituents. We hope to examine extraction cartridges filled with a form of styrene divinylbenzene, similar to the XAD-2, -4, and -16 resins that have been useful in the past for extracting phenolics and color. These have only recently become available, and may prove to be the method needed to supplement the SAX information, thereby obviating the need to perform liquid/liquid extractions in the future.

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Table 1. Comparison of SAX extraction and acidified methanol/ethyl acetate procedure on selected compounds in two raw sugars. (Reported as ppm on sugar.)

Compound	Mozambique raw		Fiji raw	
	MeOH/EtAC	SAX	MeOH/EtAC	SAX
Malonic	3.14	0.85	2.18	0.50
Succinic	5.29	3.08	2.00	1.38
Glyceric	2.35	1.20	0.71	2.63
Malic	6.33	15.74	5.07	8.69
Aspartic	0.11	5.51	0	2.71
5-oxo-proline	1.40	2.95	2.23	2.52
Citric	Fru interferes	18.07	Fru interferes	11.43
Aconitic	19.07	3.88	45.9	5.77
Quinic	1.46	53.25	1.67	23.40
Palmitic	2.50	1.29	0.76	1.28
Stearic	1.59	3.65	0.60	2.92
Glucono-delta-lactone	0	11.84	0	2.91
Gluconic acid	0.22	25.99	0	6.87

Table 2. Constituents found in two cane juice varieties.

Compound	ppm on cane juice solids	
	Variety CP 72-370	Variety CP 70-321
Phosphoric acid	126	132
Malic acid	19	20
Aconitic acid	305	316
Quinic acid	75	82

Table 3. Compounds isolated from a standard beet liquor that produced a high color white sugar. (Results reported as ppm on solids.)

R.T.	Compound	ppm
7.45	HMF	4.2
8.69	p-Hydroxybenzaldehyde	0.009
11.98	Vanillin	0.008
12.09	Cinnamic acid	0.01
15.18	Syringaldehyde	0.009
16.28	Vanillic acid	0.005
18.96	Isoferulic acid	0.022
19.16	p-Hydroxycinnamic acid	0.011
20.84	Palmitic acid	0.46
21.60	Ferulic acid	0.06
23.32	C18 acid	0.87
23.40	C18 acid	0.29
23.67	Stearic acid	0.03
25.75	HMF dimer	0.20
29.71	DHL-I	0.038
30.06	DHL-II	0.017
30.38	DFA-I	0.022

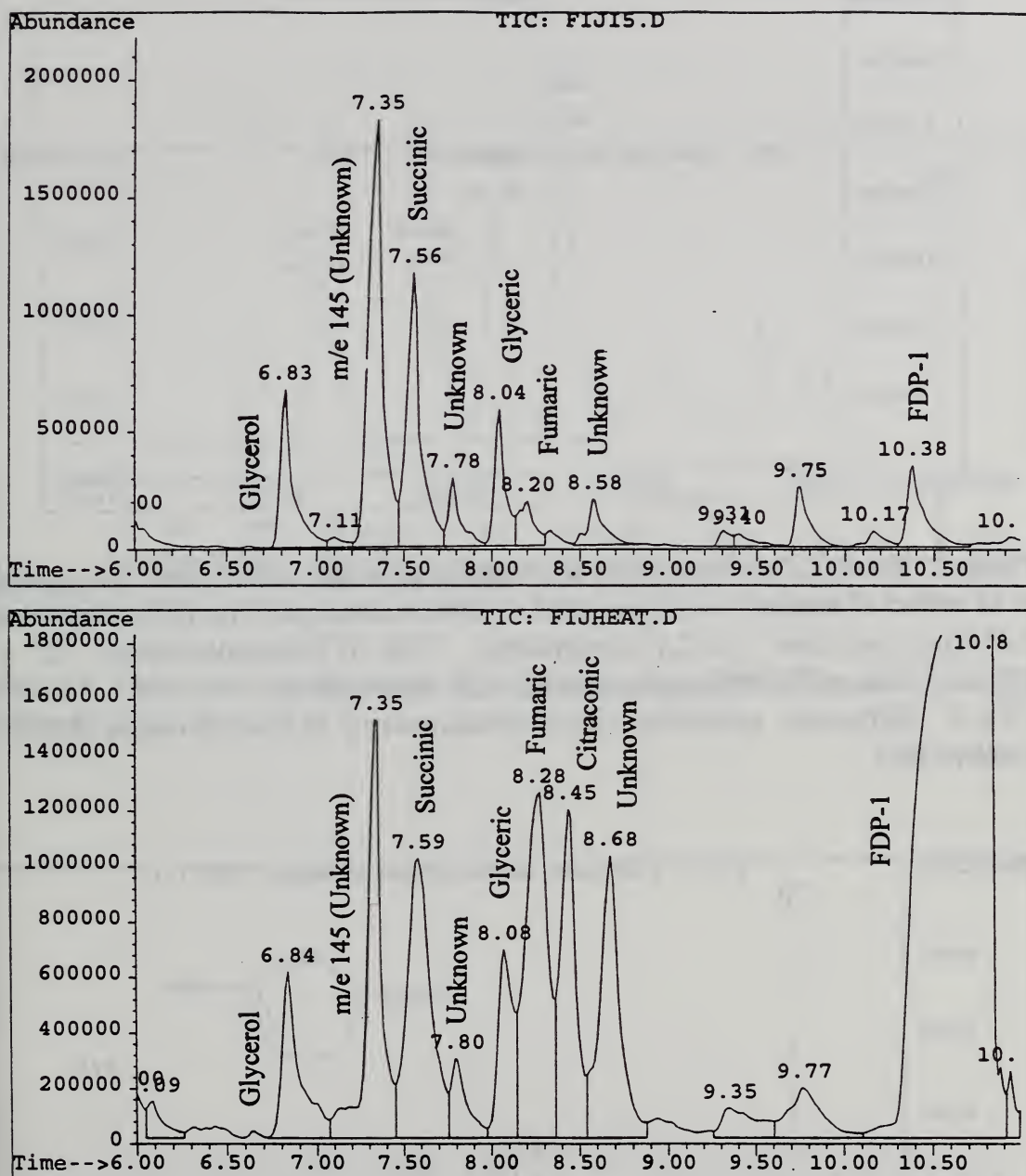


Figure 1. Portion of chromatogram showing the effect of heating a Fiji raw sugar (bottom), compared to the unheated sugar (top). The change in retention time of the fructose degradation product (FDP-1) is due to overloading of the column. Refer to Table A-3 for quantities extracted.

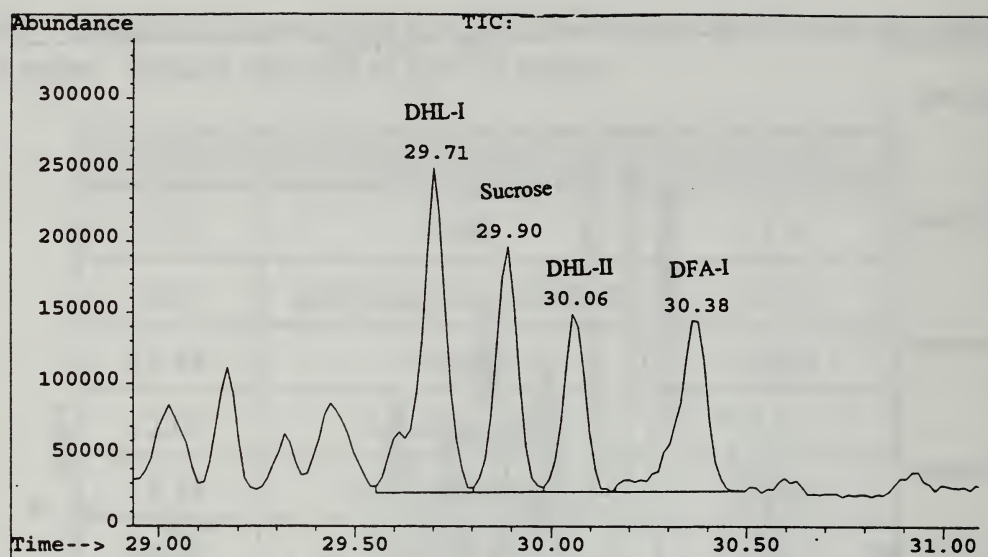


Figure 2. Portion of chromatogram showing the presence of difructose dianhydrides in an extract of standard beet liquor. DHL-I: Diheterolevulosan I (α -D-fructopyranose- β -D-fructopyranose 1,2':2,1'-dianhydride). DHL-II: Diheterolevulosan II (α -D-fructofuranose- β -D-fructopyranose 1,2':2,1'-dianhydride). DFA-I: Difructose anhydride (α -D-fructofuranose- β -D-fructofuranose 1,2':2,1'-dianhydride)

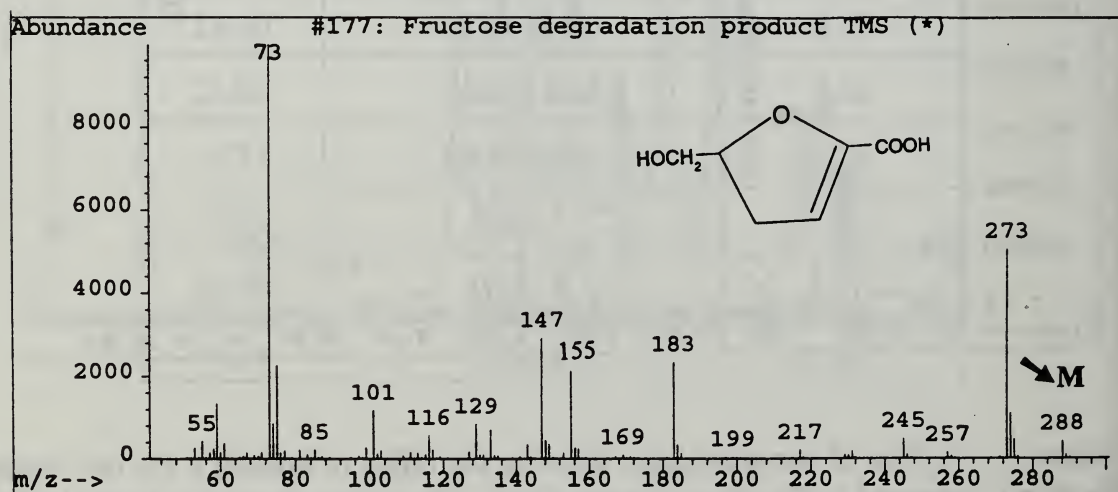


Figure 3. Mass spectrum of fructose degradation product (FDP-1) found in raw sugar colorant.

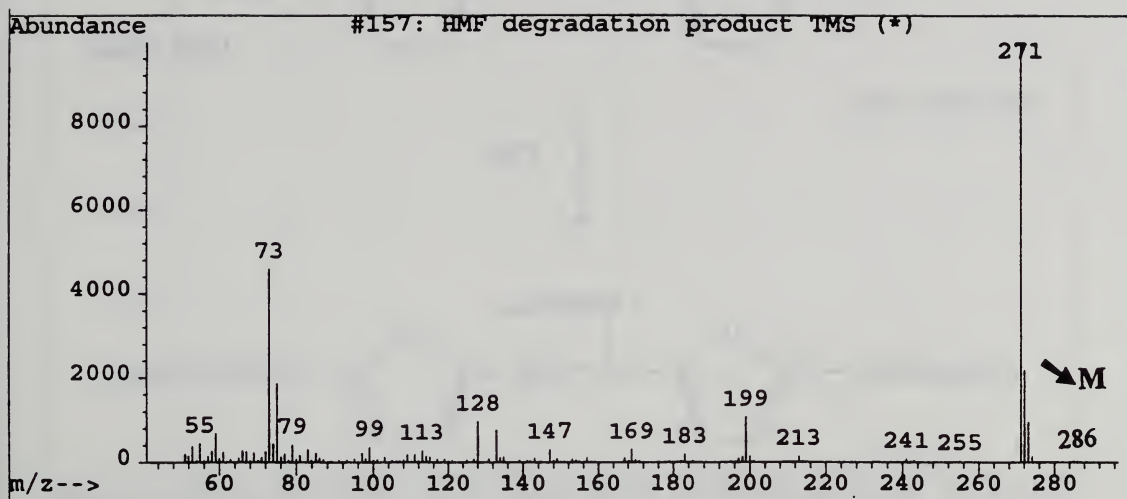


Figure 4. Mass spectrum of fructose degradation product (FDP-2) found in raw sugar colorant.

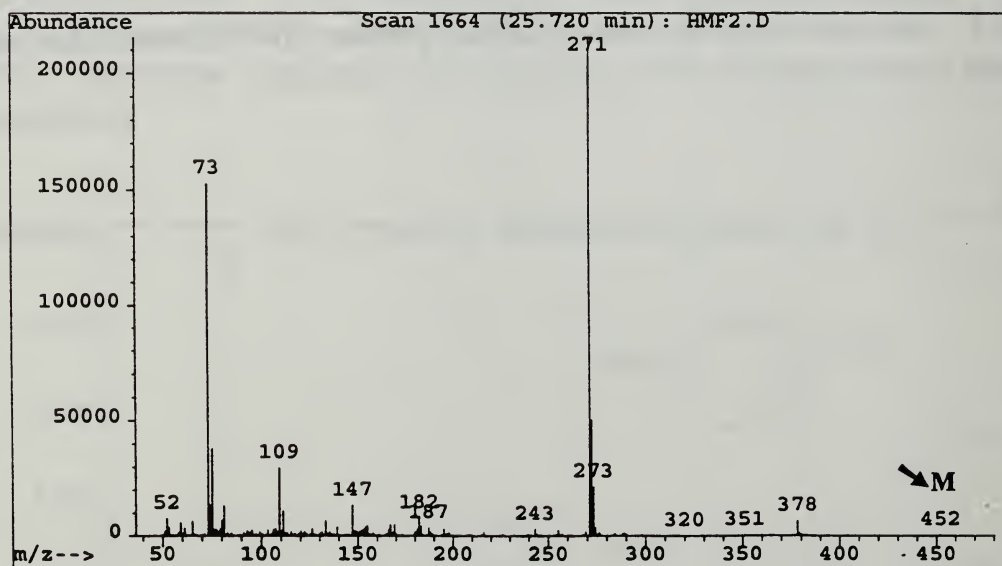
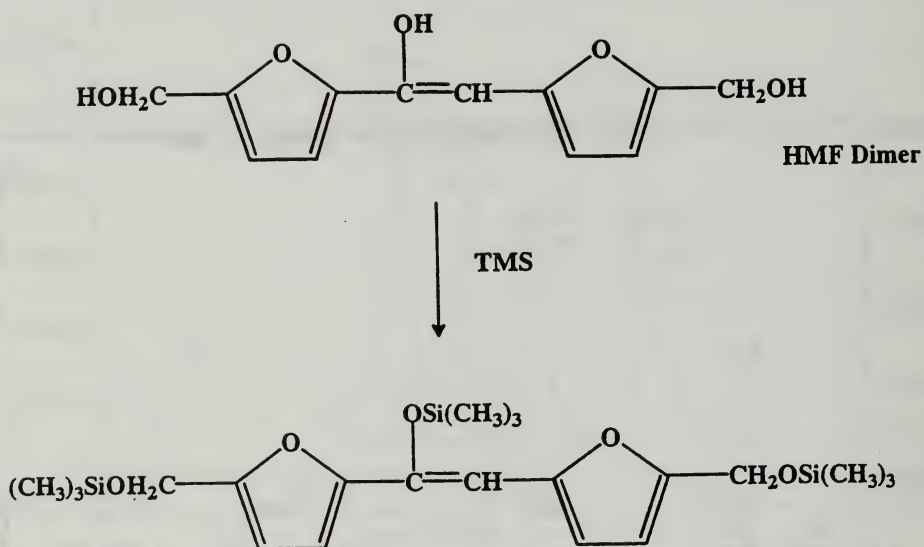


Figure 5. Top: Proposed struct of HMF dimer found in some beet and cane sugar colorant extracts. Bottom: Mass spectrum of the TMS derivative of HMF dimer.

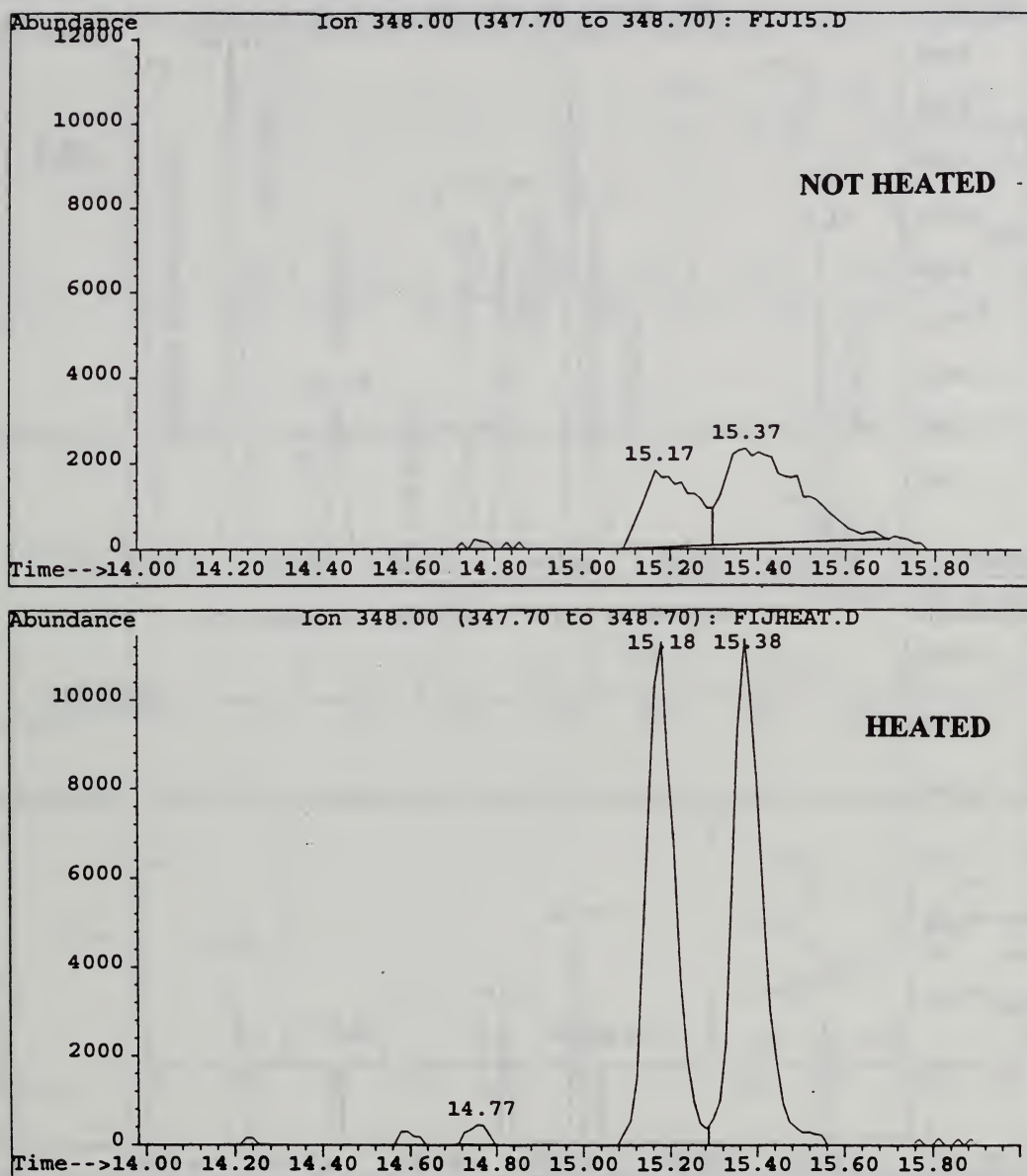


Figure 6. Chromatograms of mass ion m/z 348, showing the increase in two tentatively identified lactones in heated Fiji raw sugar (bottom) compared to the unheated sugar (top). The lactones are tentatively identified as isomers of 3-deoxy-2-C-hydroxymethyl-D-erythro-pentonic acid gamma lactone.

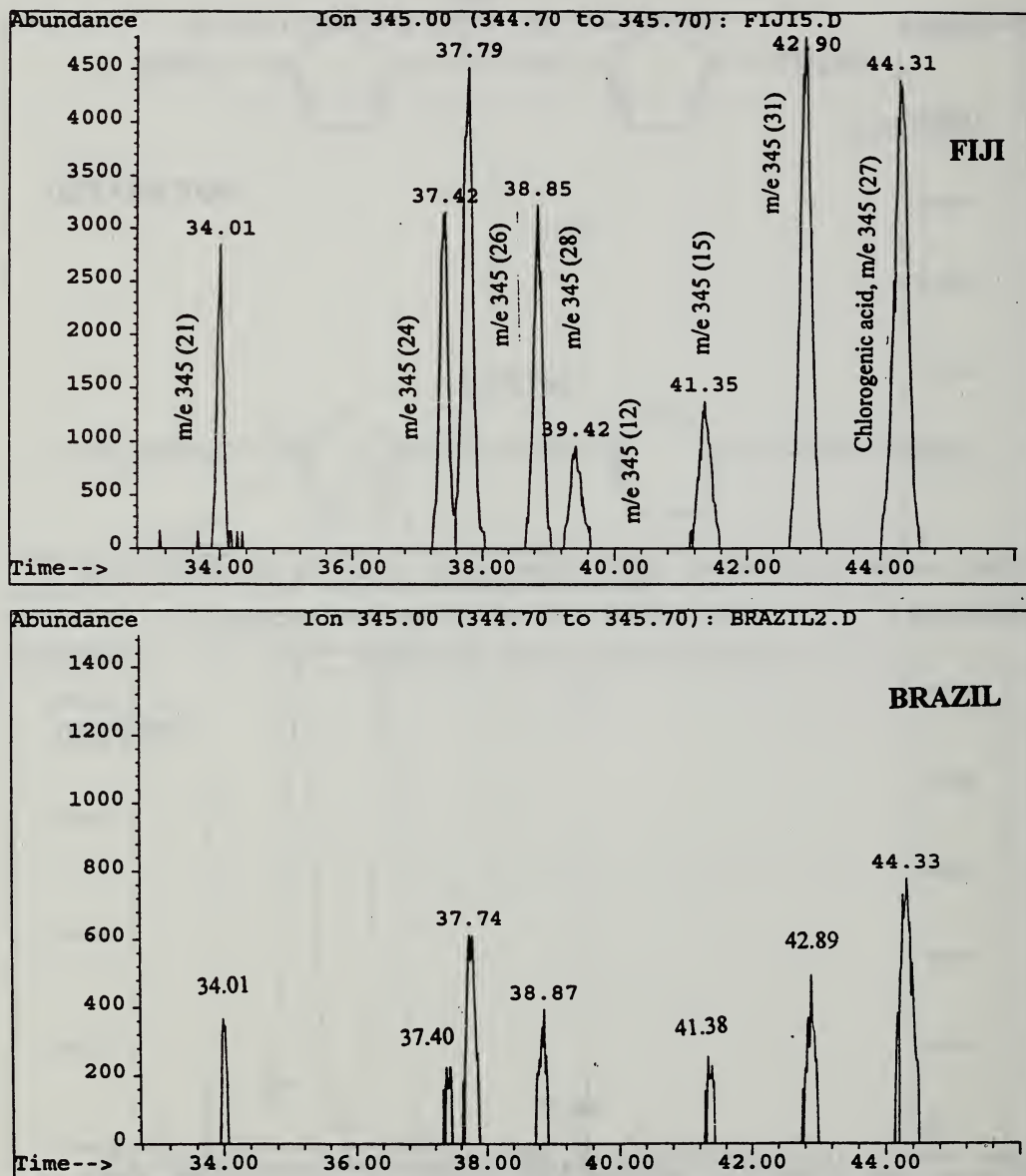


Figure 7. Chromatograms of mass ion m/z 345, characteristic of quinic acid and its esters. Top: Fiji raw. Bottom: Brazil raw. Figures in parentheses represent the relative abundance of m/z 345.

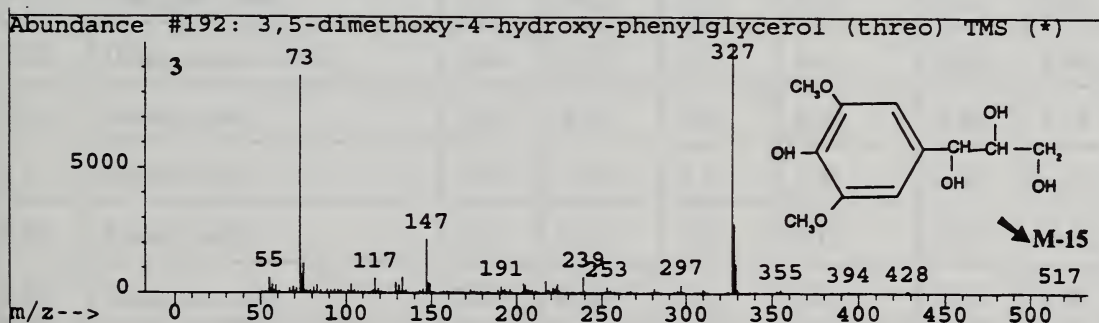
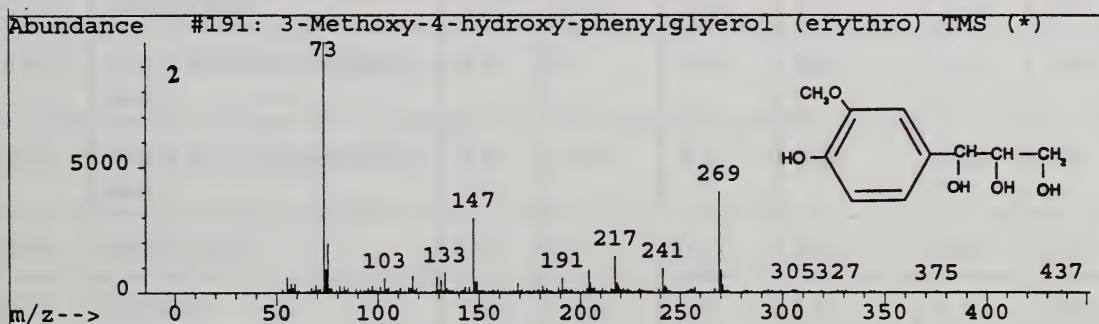
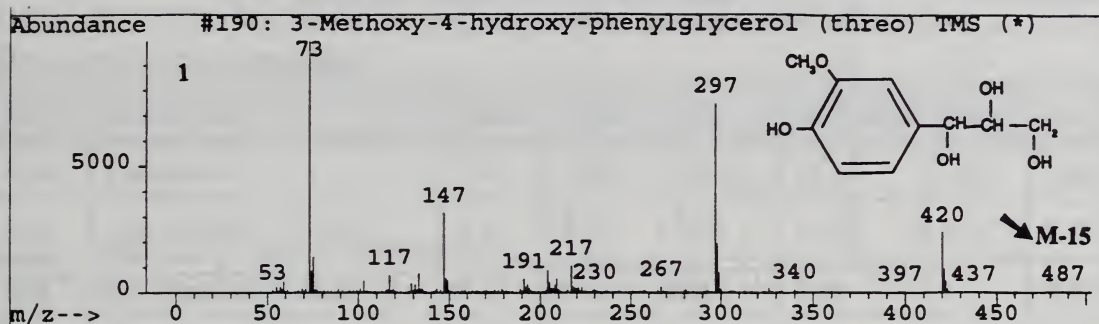


Figure 8. Structures and mass spectra of 3 tentatively identified phenyl glycerols.

- (1) 3-methoxy-4-hydroxy phenyl glycerol (*threo*)
- (2) 3-methoxy-4-hydroxy phenyl glycerol (*erythro*)
- (3) 3,5-dimethoxy-4-hydroxy phenyl glycerol (*threo*)

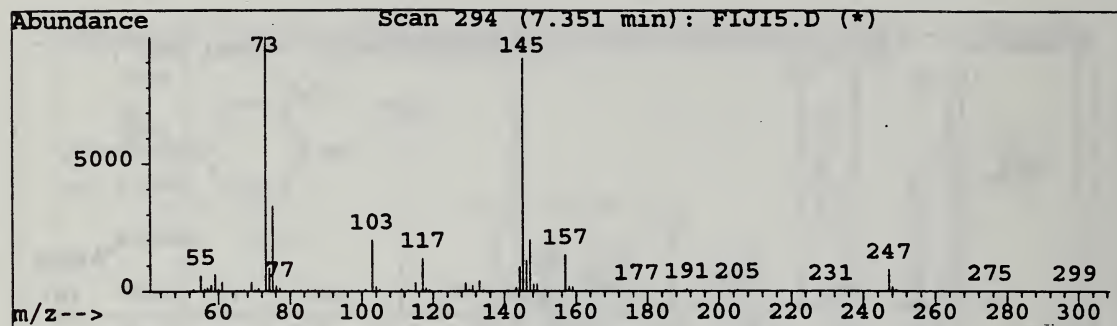


Figure 9. Mass spectrum of unknown compound eluting at 7.35 min.

APPENDIX - Tables A-1, A-2, and A-3

Table A-1. Components in raw sugars by methanol/ethyl acetate extraction.
(Reported as ppm on sugar)

R.T.	Compound	Guate	Brazil	Fiji	Mozamb	D.R.	La.
3.50	Lactic acid	7.44	16.78	18.02	21.64	9.98	8.69
3.85	Unk (m/z 73, 147, 205)	2.97	8.27	2.92	7.44	4.46	2.78
4.04	2-furancarboxylic acid	0.23	0.30	0.13	0.15	0.15	0.37
4.22	3-OH-propanoic acid	1.23	4.23	0.70	2.27	1.78	0.89
5.38	Malonic acid	1.65	0.15	2.18	3.14	2.20	2.49
5.96	3-or-4-Me-2-OH-pentanoic acid	0.14	0	0.08	0.08	0.14	0.40
6.02	3-or-4-Me-2-OH-pentanoic acid	0.19	0.69	0.13	0.26	0.15	0.51
6.08	Benzoic acid	0.15	0	0.12	0.24	trace	0.46
6.85	Glycerol	1.32	2.49	1.01	1.61	1.12	0.87
6.90	Phosphoric acid	0.13	trace	0.10	0.16	0.11	0.09
7.35	Unknown (m/z 145)	2.39	2.93	3.17	1.01	3.04	2.96
7.63	Succinic acid	3.07	4.67	2.00	5.29	2.68	4.14
8.11	Glyceric acid	1.28	2.98	0.71	2.35	1.28	0.55
8.22	Fumaric acid	0.55	0.18	0.35	0.18	0.30	1.92
8.36	Citraconic acid	0.29	0.004	0.13	0.09	0.09	0.98
9.34	Resorcinol	0.17	0.33	0.09	0.53	0.16	0.21
10.38	Fructose degrd. prod. (m/z 273) (FDP-1)	0.38	0.33	0.68	0	0.66	1.47
11.10	Fructose degrd. prod. (m/z 271) (FDP-2)	trace	0.09	0.03	0	trace	0
11.50	Malic acid	6.11	4.77	5.07	6.33	6.17	9.22
11.52	Lactic acid lactate	0.30	0.56	0.17	1.40	0.33	0

R.T.	Compound	Guatc	Brazil	Fiji	Mozamb	D.R.	La.
11.71	Lactone (m/z 147, 189, 261)	1.41	3.29	0.63	1.80	2.16	0
11.91	5-oxo-Proline	1.62	1.34	2.23	1.40	1.14	4.01
12.49	5-OHMe-2-furan- carboxylic acid	0.45	2.51	0.25	0.48	0.51	0.26
12.60	Erythronic acid	0.34	0.72	0.58	0.16	0.23	trace
12.91	C4 acid	0.19	0.21	0.13	trace	0.04	trace
13.80	p-OH-Benzoic acid	0.56	0.84	0.87	1.17	1.00	1.58
14.03	3-OH-Benzeneacetic	0.08	0.11	0	0.17	0.10	0
14.14	Xylonic acid lactone	0	0.86	0.20	0.37	0.42	0
14.20	2-C-Me-Ribonic acid lactone	0	0	1.40	0	0	0
16.38	Vanillic acid	*	0.90	*	*	*	*
16.90	Aconitic acid	33.04	2.54	45.9	19.07	8.30	86.78
17.46	3,4-di-OH-benzoic	1.35	2.58	trace	1.90	trace	trace
18.04	Citric acid	2.90	trace	**	**	**	3.81
18.50	Quinic acid	1.12	1.63	1.67	1.46	1.65	3.20
18.73	Syringic acid	1.19	1.56	3.18	1.81	2.34	2.61
19.27	p-OH-Cinnamic acid	0.49	0.15	1.32	0.42	0.97	2.82
19.48	Cetyl alcohol	0.34	trace	0.41	trace	0.17	0.42
19.77	3,4,5-tri-OH-Benzoic	0.05	0.09	0	0	0.10	0.28
20.57	Unknown (m/z 287)	0.25	0	0	0	0.24	0.19
20.71	Gluconic acid	0.13	0.09	0	0.22	trace	trace
20.83	Unknown (m/z 297) ⁽¹⁾	trace	trace	0.36	trace	trace	0.91
20.87	Palmitic acid	1.95	2.54	0.76	2.50	1.35	1.64
21.41	Unknown (m/z 269) ⁽²⁾	0.14	0.14	0.13	trace	0.21	0.70

R.T.	Compound	Guat	Brazil	Fiji	Mozamb	D.R.	La
21.64	Ferulic acid	0.08	0.08	0.26	0.15	0.35	0.88
22.12	Unknown (m/z 327) ⁽³⁾	0.05	0.24	0.16	trace	0.23	0.70
22.38	Caffeic acid	0.03	trace	trace	trace	0.23	0.90
22.46	Unknown (m/z 253,327,355, 370)	0.28	0.46	0.90	0.76	0.65	0.98
23.30	9,12-octadecadienoic	1.63	1.46	0.31	0.81	0.63	0.87
23.37	Oleic acid	0.83	0.70	0.30	0.63	0.39	0.64
23.72	Stearic acid	0.60	1.26	0.60	1.59	0.61	0.76
24.85	Unknown (m/z 267) ⁽⁴⁾	0.11	trace	0.34	trace	0.29	0.59
25.18	Unknown (m/z 267) ⁽⁴⁾	0.25	trace	0.47	trace	0.26	1.14
25.71	Unknown (m/z 239)	0.10	0	0	trace	0.09	0.57
25.94	Hexanedioic acid ester	4.23	0.11	0	trace	3.97	2.80
25.95	Phosphoric acid, tri-phenyl ester (m/z 326)	0	0.11	0.74	1.18	0	0
27.78	Phthalate	2.54	1.00	2.08	0.47	2.37	2.12
35.29	Unknown (m/z 439)	0.11	0.05	0	0.14	0.09	1.27
35.73	Glucoside of (1) (m/z 297)	trace	trace	0.16	trace	0.13	0.49
38.03	Glucoside of (1) (m/z 297)	trace	trace	0.23	trace	0.41	0.37
40.98	Glucoside of (3) (m/z 327)	trace	0.02	0.32	trace	0.22	0.16
41.22	Glucoside of (3) (m/z 327)	0.11	0.01	0.11	trace	0.25	0.07
42.62	Unknown (m/z 467)	0.66	0.24	0.20	0.32	0.47	2.44
42.86	Chlorogenic isomer	0.18	0.03	0.27	0.08	0.52	0.51
44.29	Chlorogenic acid	0.27	0.07	0.27	0.24	0.32	1.41

* Interference by aconitic acid

** Interference by fructose

⁽¹⁾ Tentatively identified as 3-methoxy-4-hydroxy-phenyl-glycerol aglycone (*threo*-form) (Palla, 1983)

⁽²⁾ Tentatively identified as 3-methoxy-4-hydroxy-phenyl-glycerol aglycone (*erythro*-form) (Palla, 1983)

⁽³⁾ Tentatively identified as 3,5-dimethoxy-4-hydroxy-phenyl-glycerol aglycone (Palla, 1983)

⁽⁴⁾ Structurally similar to 4-hydroxy-phenyl-(α -hydroxy) acetic acid

Table A-2. Comparison of various extraction procedures on amount of components extracted from a Fijian raw sugar. (Reported as ppm on sugar)

R.t.	Compound	MeOH/EtAc, acidified	MeOH/EtAc not acidified	EtAc/H ⁺ liq/liq	SAX cartri dge	C18 cartri dge
3.50	Lactic acid	18.02	1.98	22.29	55.45	7.36
3.85	Unk (m/z 73,147,205)	2.92	0.51	1.19	10.50	0.59
4.04	2-furancarboxylic acid	0.13	0	0.06	0	0
4.22	3-OH-propanoic acid	0.70	0.15	0.55	1.87	0
5.38	Malonic acid	2.18	0.02	0.66	0.50	0.064
5.96	3-or-4-Me-2-OH- pentanoic acid	0.08	0.006	0.14	0	0.04
6.02	3-or-4-Me-2-OH- pentanoic acid	0.13	0.004	0.14	0	0.04
6.08	Benzoic acid	0.12	0.009	0.12	trace	0.04
6.85	Glycerol	1.01	0.92	0.37	1.28	0
6.90	Phosphoric acid	0.10	trace	trace	1.73	1.96
7.35	Unknown (m/z 145)	3.17	2.61	6.19	0	0.11
7.63	Succinic acid	2.00	0.14	1.26	1.38	0.08

R.t.	Compound	MeOH/EtAc, acidified	MeOH/EtAc not acidified	EtAc/H ⁺ liq/liq	SAX cartri dge	C18 cartrid ge
8.11	Glyceric acid	0.71	0.05	0.37	2.63	0.07
8.22	Fumaric acid	0.35	trace	0.41	trace	trace
8.36	Citraconic acid	0.13	0	0.32	0	0
9.34	Resorcinol	0.09	0.10	0.30	0	0
10.38	Fructose degrd. prod. (m/z 273) (FDP-1)	0.68	0.64	1.53	0	trace
11.10	Fructose degrd. product (m/z 271) (FDP-2)	0.03	0	0	0	0
11.50	Malic acid	5.07	0.13	4.77	8.69	0.07
11.52	Lactic acid lactate	0.17	0.14	0.71	trace	0
11.71	Lactone (m/z 147, 189, 261)	0.63	0.60	0.65	0	0
11.91	5-oxo-Proline	2.23	0.02	1.24	2.52	0.04
11.94	Aspartic acid	0	0	0	2.71	0
12.49	5-OHMe-2-furan- carboxylic acid	0.25	0	0.85	0	0
12.60	Erythronic acid	0.58	0.007	0.03	2.28	0.04
12.91	C4 acid	0.13	0	0	0.27	0
13.80	p-OH-Benzoic acid	0.87	0.58	1.31	0	0
14.03	3-OH-Benzeneacetic	0	0	0	0	0
14.14	Xylonic acid lactone	0.25	0	0	0.67	0
14.20	2C-Me-Ribonic acid lactone	1.40	0.58	0	1.51	0
16.38	Vanillic acid	*	0.61	*	0	0.09
16.90	Aconitic acid	45.9	0.88	56.76	5.77	0.25

R.t	Compound	MeOH/EtAc, acidified	MeOH/EtAc not acidified	EtAc/H ⁺ liq/liq	SAX cartri dge	C18 cartrid ge
17.46	3,4-di-OH-benzoic	trace	trace	trace	0	0
18.04	Citric acid	**	0	**	11.43	0
18.50	Quinic acid	1.67	1.06	0.24	23.40	0.19
18.72	Gluconolactone	0	0	0	2.91	0
18.73	Syringic acid	3.18	1.50	0.68	0	0.66
19.27	p-OH-Cinnamic acid	1.32	1.08	1.37	0	0.46
19.48	Cetyl alcohol	0.41	0.23	0.59	0	0.11
19.77	3,4,5-tri-OH- Benzoic	0	0	0	0	0
20.57	Unknown (m/z 287)	0	0	0	0	0
20.71	Gluconic acid	0	0	0	6.87	0
20.83	Unknown (m/z 297)	0.36	0.34	0	0	trace
20.87	Palmitic acid	0.76	0.76	0.86	1.28	0.88
21.41	Unknown (m/z 269)	0.13	0.22	0	0	trace
21.64	Ferulic acid	0.26	0.39	0.45	0	0.13
22.12	Unknown (m/z 327)	0.16	0.30	0.34	trace	0.25
22.38	Caffeic acid	trace	0	0	0	0
22.46	Unk (m/z 253,327,355,370)	0.90	0.77	0.79	0	0.13
23.30	9,12- octadecadienoic	0.31	0.29	0.48	0.12	0.13
23.37	Oleic acid	0.60	0.58	0.40	0.16	0.15
23.72	Stearic acid	0.47	1.12	0.38	2.92	1.53
24.85	Unknown (m/z 267)	0.34	0.02	0.71	0	0

R.t	Compound	MeOH/EtAc, acidified	MeOH/EtAc not acidified	EtAc/H ⁺ liq/liq	SAX cartri dge	C18 cartrid ge
25.18	Unknown (m/z 267) (¹)	0.47	trace	0.65	0	trace
25.71	Unknown (m/z 239)	0	0	0	0	0
25.94	Hexanedioic acid ester	0	0	0	0	trace
25.95	Phosphoric acid triphenyl ester (m/z 326)	0.74	0.56	0	0	0
27.78	Phthalate	2.08	2.70	1.41	9.03	1.61
35.29	Unknown (m/z 439)	0	trace	0	0	0
35.73	Glucoside of (1) (m/z 297)	0.16	0.02	0.21	0	trace
38.03	Glucoside of (1) (m/z 297)	0.23	0.08	0.33	0	trace
40.98	Glucoside of (3) (m/z 327)	0.32	0.08	0.40	trace	trace
41.22	Glucoside of (3) (m/z 327)	0.11	trace	0.10	trace	trace
42.62	Unknown (m/z 467)	0.20	trace	0	0	0
42.86	Chlorogenic isomer	0.27	trace	0.06	0	0.23
44.29	Chlorogenic acid	0.27	trace	0.06	0	trace

* Interference by aconitic acid

** Interference by fructose

C18 also extracted the steroids, stigmasterol and sitosterol.

(¹) Tentatively identified as 3-methoxy-4-hydroxy-phenyl-glycerol aglycone (*threo*-form) (Palla, 1983)

(²) Tentatively identified as 3-methoxy-4-hydroxy-phenyl-glycerol aglycone (*erythro*-form) (Palla, 1983)

⁽³⁾ Tentatively identified as 3,5-dimethoxy-4-hydroxy-phenyl-glycerol aglycone (Palla, 1983)

⁽⁴⁾ Structurally similar to 4-hydroxy-phenyl-(α -hydroxy) acetic acid

Table A-3. Comparison of components found in a methanol/ethyl acetate extract of unheated and heated Fijian raw sugar. (Reported as ppm)

R.t	Compound	Not heated	Heated
3.50	Lactic acid	18.02	20.25
3.85	Unk (m/z 73,147,205)	2.92	11.56
4.04	2-furancarboxylic acid	0.13	0.58
4.22	3-OH-propanoic acid	0.70	1.65
5.38	Malonic acid	2.18	1.40
5.96	3-or-4-Me-2-OH-pentanoic acid	0.08	0.22
6.02	3-or-4-Me-2-OH-pentanoic acid	0.13	--
6.08	Benzoic acid	0.12	0.11
6.85	Glycerol	1.01	1.65
6.90	Phosphoric acid	0.10	0.10
7.35	Unknown (m/z 145)	3.17	3.56
7.63	Succinic acid	2.00	3.90
8.11	Glyceric acid	0.71	1.59
8.22	Fumaric acid	0.35	3.25
8.36	Citraconic acid	0.13	2.96
9.34	Resorcinol	0.09	0.61
10.38	Fructose degrd. product (m/z 273) (FDP-1)	0.68	37.17
11.10	Fructose degrd. product (m/z 271) (FDP-2)	0.03	0.48
11.50	Malic acid	5.07	5.25

R.t.	Compound	Not heated	Heated
11.52	Lactic acid lactate	0.17	1.41
11.71	Lactone (m/z 147, 189, 261)	0.63	4.16
11.91	5-oxo-Proline	2.23	3.18
11.94	Aspartic acid	0	0
12.49	5-OHMe-2-furan-carboxylic acid	0.25	1.23
12.60	Erythronic acid	0.58	0.51
12.91	C4 acid	0.13	0.15
13.80	p-OH-Benzoic acid	0.87	1.85
14.03	3-OH-Benzeneacetic	0	0
14.14	Xylonic acid lactone	0.25	1.25
14.20	2C-methyl ribonic acid lactone	1.40	2.32
16.38	Vanillic acid	*	*
16.90	Aconitic acid	45.9	10.60
17.46	3,4-di-OH-benzoic acid	trace	trace
18.04	Citric acid	**	**
18.50	Quinic acid	1.67	2.90
18.72	Gluconolactone	0	0
18.73	Syringic acid	3.18	1.77
19.27	p-OH-Cinnamic acid	1.32	0.73
19.48	Cetyl alcohol	0.41	0.58
19.77	3,4,5-tri-OH-Benzoic	0	0
20.57	Unknown (m/z 287)	0	0.44
20.71	Gluconic acid	0	0
20.83	Unknown (m/z 297) ⁽¹⁾	0.36	trace
20.87	Palmitic acid	0.76	1.12

R.t.	Compound	Not heated	Heated
21.41	Unknown (m/z 269) ⁽²⁾	0.13	trace
21.64	Ferulic acid	0.26	0.16
22.12	Unknown (m/z 327) ⁽³⁾	0.16	0.02
22.38	Caffeic acid	trace	0
22.46	Unk (m/z 253, 355, 370)	0.90	1.20
23.30	9,12-octadecadienoic	0.31	2.23
23.37	Oleic acid	0.60	0
23.72	Stearic acid	0.47	1.52
24.85	Unknown (m/z 267) ⁽⁴⁾	0.34	0.31
25.18	Unknown (m/z 267) ⁽⁴⁾	0.47	0.21
25.71	Unknown (m/z 239)	0	0
25.94	Hexanedioic acid ester	0	1.57
25.95	Phosphoric acid triphenyl ester (m/z 326)	0.74	0
27.78	Phthalate	2.08	0
35.29	Unknown (m/z 439)	0	0
35.73	Glucoside of (1) (m/z 297)	0.16	0.02
38.03	Glucoside of (1) (m/z 297)	0.23	0.21
40.98	Glucoside of (3) (m/z 327)	0.32	0.11
41.22	Glucoside of (3) (m/z 327)	0.11	trace
42.62	Unknown (m/z 467)	0.20	0.23
42.86	Chlorogenic isomer	0.27	0.25
44.29	Chlorogenic acid	0.27	0.24

* Interference by aconitic acid

** Interference by fructose

- (1) Tentatively identified as 3-methoxy-4-hydroxy-phenyl-glycerol aglycone (*threo*-form) (Palla, 1983)
- (2) Tentatively identified as 3-methoxy-4-hydroxy-phenyl-glycerol aglycone (*erythro*-form) (Palla, 1983)
- (3) Tentatively identified as 3,5-dimethoxy-4-hydroxy-phenyl-glycerol aglycone (Palla, 1983)
- (4) Structurally similar to 4-hydroxy-phenyl-(α -hydroxy) acetic acid

DISCUSSION

Question: If we have problems with raw sugars, should we do these extractions and send you material off the column so you could do the identification for us?

Godshall: Yes, certainly. Part of our research program is colorant formation, and part of our overall service to our sponsors is to help sponsors understand and solve their color problems.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPIC STUDIES OF POLYSACCHARIDES IN SUGARCANE PROCESSING

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ABSTRACT

Nuclear magnetic resonance (nmr) spectroscopy is an extensively used, sophisticated tool for chemical structure elucidation and quantitative analysis. In this presentation some potential applications of nmr spectroscopy for problem solving in the sugar industry will be discussed. Molasses and sugar samples collected during the 1995 Louisiana cane sugar campaign have been fractionated by several methods and the chemical structure of the high molecular weight materials have been studied. In addition nmr spectroscopic methods offer a new and more rigorous approach to quantitative analysis of polysaccharides in raw sugars (*viz.*, starch and dextran). This presentation also examines the quantitative analysis of dextran in raw sugar by ^1H nmr spectroscopy.

INTRODUCTION

The use of high field nmr spectroscopy for chemical structure elucidation and quantitative analysis is well established. It has become an indispensable tool for research in chemistry, biochemistry, physics and recently magnetic resonance tomography or imaging has become an important investigative tool in medicine. The nmr methods have become extremely sophisticated, but even at a simple level nmr has many potential applications to problem solving in the sugar industry. For example, inexpensive low field nmr instruments may have application to the measurement of sucrose crystal contents in massecuites (1), and high field nmr has been used to quantitatively determine dextran concentration in raw sugars (2, 3). This paper reviews the development of the quantitative ^1H nmr spectroscopic analysis of polysaccharides in raw sugars (work undertaken at SPRI in the last three years) and presents recent results from a study on the fate of polysaccharides and colorants in sugarcane processing.

Quantitative analysis of dextran and starch in raw sugars

Dextrans are a class of extracellular microbial polysaccharides consisting of a backbone of α -D-glucopyranosyl residues with (1-6) linkages. Naturally occurring dextrans usually contain (1-3) branch points and sometimes (1-2) or (1-4) branch points (4). The infection of sugarcane and cane milling streams with dextran forming bacteria is of concern to the sugar industry. The microbial biosynthesis of dextrans occurs via the action of extracellular dextranase enzymes on sucrose. Consequently, infections of sugarcane and cane milling processing streams with these bacteria cause loss of sucrose to dextran. Furthermore, high dextran concentrations in process streams effect an increase in viscosity that leads to reduced recovery of sucrose and reduced factory efficiency. Finally, dextrans and α -glucan oligosaccharides (the products of dextran hydrolysis) are dextrorotatory and their presence in raw sugar at the point of sale to refiners increases the polarimetric measurement of sucrose purity.

Previously, we have reported a physical measurement for quantitative analysis for dextran based on anomeric proton signals in ^1H nmr spectra of raw sugar solutions (2, 3). The primary intent was to establish a reference method (rather than a routine analysis) by which the methods currently favored by the sugar industry (*viz.*, haze (5) and AOAC (6) methods) could be compared. The discrepancies between the haze and AOAC methods are due to differences in specificity and sensitivity of the chemical analyses. The haze method involves enzymic removal of high molecular weight starch, ion-exchange removal of inorganic salts (in some versions), precipitation of proteins with trichloroacetic acid and measurement of turbidity of a 50% aqueous ethanol solution. The haze method is not sensitive at low dextran concentrations and is specific for high molecular weight, relatively linear dextran (*i.e.*, dextran that precipitates in 50% aqueous ethanol). The AOAC method involves quantitative precipitation of total polysaccharides in 80% aqueous ethanol; the precipitate is redissolved and selective precipitation of dextrans in alkaline copper solution is followed by colorimetric determination of sugars with the phenol-sulfuric acid reagent. While the haze method is specific for high molecular weight dextran, the AOAC method appears to be not specific to a molecular weight range. Hence, the AOAC method results are usually significantly higher than those of the haze test. In addition to the total dextran, the copper precipitate of the AOAC method may contain 1 to 4% non-dextran polysaccharides, as does the alcohol precipitate in the haze test.

The nmr spectroscopic method of analysis of dextran involves the use of stractan (a water soluble polysaccharide with molecular weight >10,000 Da and no ^1H nmr signals that overlap in the anomeric proton region with either dextran or sucrose) as an internal standard so that dextran concentration in the high molecular weight fraction of a raw sugar solution can be related to dextran concentration in the raw sugar. The ^1H nmr spectrum of stractan shows a major signal in the anomeric proton region (4.46 ppm, d, $J = 8.3$ Hz) due to the C1-H of β -(1-3) linked D-galp; other minor peaks do not overlap with the dextran anomeric proton signals. Raw sugars are dissolved in aqueous solutions spiked with dextran and stractan (in varying proportions) and the high molecular weight fractions are concentrated by membrane filtration (10,000 MWCO). Figure 1 is a ^1H nmr spectrum (500 MHz, expanded to show the anomeric proton signals of interest) of a raw sugar solution spiked with stractan and dextran and after concentration by membrane filtration. For each raw sugar a plot of ^1H nmr peak area ratios of the anomeric proton signals (dextran (4.98 ppm) and stractan (4.46 ppm)) versus weight ratios of added dextran/stractan is a straight line offset from a zero y-axis intercept by the initial amounts of dextran in the raw sugars.

The results of the determination of dextran concentration in six raw sugars by the ^1H nmr method are compared to the results of the haze and AOAC methods in Table 1. The nmr method is more rigorous than the haze or AOAC methods since it obtains a quantitative measurement of all anomeric protons of α -D-glucopyranosyl residues with (1-6) linkages (*i.e.*, all dextran regardless of solubility in aqueous ethanolic solutions, molecular weight or degree of branching). It would appear that, at least in these six samples, the haze method underestimates dextran content in raw sugars, and that the AOAC method is in reasonable agreement with the ^1H nmr method.

It also is possible to quantitatively measure starch in raw sugars by the same methodology. However, the 500 MHz nmr instrument, operator, and maintenance costs mitigate against routine use of this method in the sugar industry. We do not suggest that ^1H nmr methods should replace existing methods for the determination of polysaccharides in raw sugars. The primary intent in the development of the dextran-nmr analysis was to establish a reference method by which the haze and AOAC methods could be compared. Since an infrequently performed, simple colorimetric blue starch-iodine complex assay appears to be uniformly adopted by the sugar industry, the development of a starch-nmr analysis is a low priority.

We also explored the differences in the conventional analytical methods (*viz.*, haze and AOAC) by the analysis of alcoholic precipitates of raw sugar solutions using gel permeation chromatography (GPC) and ^1H nmr spectroscopy (3). In "parallel" ethanol precipitation experiments polysaccharides in raw sugar solutions (60% w/v) were precipitated at 50%, 70% and 85% v/v ethanol. In "serial" ethanol precipitation experiments polysaccharides in raw sugar solutions (60% w/v) were precipitated at 50% v/v ethanol, the precipitate was removed and more ethanol was added to the supernatant to bring the ethanol concentration to 70% v/v. A second precipitate was removed and more ethanol was added to the supernatant to bring the ethanol concentration to 85% v/v. All precipitates were separated by centrifugation and removal of the supernatant, dissolved in water (10mL), and filtered (0.45 μm) prior to GPC-HPLC injection of 0.1mL. Precipitates from 50% ethanol solution contained high molecular weight material and some sucrose, but no intermediate weight materials. Parallel precipitates from 70% and 85% ethanol contained both high and intermediate molecular weight material, the 85% ppt contained proportionally more intermediate material. The serial precipitation experiment showed that 50% ethanol precipitation (*i.e.*, the haze method) leaves behind some high molecular weight material and all intermediate weight material. Most high molecular weight material was precipitated at 70% ethanol, the remaining precipitable material (serial ppt at 85% ethanol) was mostly of intermediate weight.

The ^1H nmr spectra of these precipitates contained several doublet signals between *ca.* 4.4 to 5.7 ppm that could be tentatively assigned to the anomeric protons of sucrose, dextran and starch or possibly another polysaccharide. The average degree of branching of the dextran in these precipitates was estimated from the peak area ratio of the C1-H of α -(1 \rightarrow 6) linked D-Glc_p doublet signal (δ 4.98 ppm) and the C1-H of α -(1 \rightarrow 3) linked D-Glc_p doublet signal (δ 5.33 ppm) of dextran. The average peak area ratios from spectra where these signals were clearly resolved from baseline noise are shown in Table 2. These results confirmed that lower molecular weight dextrans in raw sugar contain more α -(1 \rightarrow 3) linked D-Glc_p branches than the higher molecular weight dextrans (*cf.*, 50 % ethanol precipitates and the 85 % ethanol serial precipitate). Therefore, the higher incidence of branching in the lower molecular weight dextrans would be a significant source of underestimation of dextran in raw sugars by enzymic methods (7-9).

These ^1H nmr and GPC studies of dextrans in raw sugars qualified the two commonly used analytical methods (*viz.*, haze test and AOAC method). While the haze test results in an underestimate of total dextrans, it is useful as a measure of processability

of a raw sugar since it is selective for high molecular weight dextran. Results from the AOAC method agreed with measurements obtained by ^1H nmr spectroscopy. The AOAC method measures total dextran including lower molecular weight α -glucans that contribute to overestimation of raw sugar sucrose content by polarimetry.

The fate of polysaccharides and colorants in sugarcane processing

Recent work on the fate of polysaccharides and colorant in sugarcane processing forms the main body of this paper. The work involves the establishment of an analytical GPC system at SPRI and the analysis of evaporator syrups, A-, B- and C-molasses and membrane filtration retentates and permeates by analytical GPC, fluorophore-assisted carbohydrate electrophoresis (FACE) and ^1H nmr.

EXPERIMENTAL - METHODS AND MATERIALS

General methods

All raw sugars were from the library of sugars at the Sugar Processing Research Institute and are representative of the range of polysaccharide concentrations found in raw sugars. Sugarcane factory evaporator syrups and A, B, and C molasses were obtained from a Louisiana factory. All other chemicals were analytical grade.

Gel permeation HPLC

The analytical gel permeation HPLC (GPC) system was a series of a guard and three TSK-gel columns (PWXL Guard, G6000PWXL, G5000PWXL and G4000PWXL [Supelco]) at ambient temperature, eluant: 0.6 mLmin^{-1} 0.2 M NaCl , 10% acetonitrile in water, and with refractive index and UV (210nm) detection. Sodium polystyrene sulfonate molecular weight standards were used to calibrate peak retention time to approximate molecular weight. Preparative fractions were collected from this column system with a Foxy Jr. fraction collector (ISCO), eluant: 1.0 mLmin^{-1} $0.2\text{ M (NH}_4)_2\text{CO}_3$, 10% acetonitrile in water, reduced to a *ca.* 3mL and dialyzed (10,000 MWCO (molecular weight cut off)). The samples were freeze dried. The ^1H and ^{13}C nmr spectra of these samples (*ca.* 4 to 10 mg dissolved in $0.75\text{ mL D}_2\text{O}$, pre-exchanged with D_2O four times) were recorded on a GE Omega series 500 MHz spectrometer (^1H at 500 MHz and ^{13}C at 125 MHz). Samples (*ca.* 1 mg) were shipped to Glyko, Inc., Novato, CA for fluorophore-assisted carbohydrate electrophoresis (FACE) analysis after acid hydrolysis (4N TFA, 100°C , 3 hr).

RESULTS AND DISCUSSION

Evaporator syrup, A-, B- and C-molasses and A sugar samples were obtained from a Louisiana sugarcane factory on a weekly basis over the entire cane harvest; the analyses of these samples are reported by Vercellotti, *et al.* (10). Three sets of weekly samples were used in this study. The gel permeation chromatograms of all three sets were essentially similar, in fact evaporator syrup, A-, B- and C-molasses also had similar profiles. The GPC of a C-molasses is shown in Figure 2, and is typical of all samples analyzed. For the purpose of this study three peaks are identified; peak 1 - high molecular weight ($> 1.2 \times 10^6$ Da), peak 2 - intermediate molecular weight (30,000 to 130,000 Da) and peak 3 - lower molecular weight material ($< 7,000$ Da). All three peaks have carbohydrate (refractive index) and colorant (UV absorption) nature. Peak 3 is complex and contains low molecular weight colored materials, sucrose, glucose, fructose and possibly other saccharides. Peaks 1 and 2 contain the soluble polysaccharides known to occur in sugarcane process streams (*e.g.*, dextran, an arabinogalactan and starch); the arabinogalactan was first described by Roberts and Godshall (SPRI) and called indigenous sugarcane polysaccharide. This study focuses on the fate in process of the polysaccharides in peaks 1 and 2.

The GPC profile in Figure 2 is obtained by elution with 0.2 M sodium chloride in acetonitrile:water (1:9). Figures 3 and 4 show the effect of eluant on the GPC profiles of evaporator syrup and C-molasses. In water and in 10% acetonitrile eluants the low molecular weight colorants are carried along with the higher molecular weight material; this is evidence for an ionic association between colorants and higher molecular weight material. The addition of sodium chloride to the eluant effects a decrease in this ionic association. Some UV absorbing compounds continue to elute with the higher molecular weight material; these are either non-carbohydrate, high molecular weight compounds or UV absorbing aglycons covalently bonded to polysaccharides. Since the UV absorption and refractive index peaks coincide the latter possibility seems more likely.

It can be seen from Figure 2 that the refractive index peaks 1 and 2 are very small, almost in electronic noise at the base line. Nevertheless, these peaks in some of the samples could be integrated. For peaks that could be integrated, the data was normalized using the sodium ion concentration (the assumption that sodium and potassium persist in the process stream from juice to C-molasses has been made by many investigators and used to normalize analytical results so that two or more

process streams can be compared). The averaged results from samples where the refractive index peaks 1 and 2 could be integrated appear in table 3. These results are strong evidence that the material in peak 1 is formed by the material in peak 2 during processing.

The high molecular weight material in evaporator syrup and C-molasses from one weekly set were concentrated by membrane filtration (10,000 MWCO) and suspended solids were removed by centrifugation. Not surprisingly, this high molecular weight fraction still contained colorant (of lower molecular weight) that could be separated from the high molecular weight material on our GPC system with elution in 0.2 M NaCl or 0.2 M $(\text{NH}_4)_2\text{CO}_3$.

The GPC-UV 210 nm profiles of these retentates were similar to the starting material; the refractive index profiles contained peaks 1 and 2 in a concentrated form but very little peak 3. Peak 2 was collected by repetitive GPC injection and collection; peak 1 was also collected but as two cuts (*viz.*, peak 1A and 1B). This collection procedure yielded mg quantities of material for each fraction. FACE analysis of these fractions from evaporator syrup and after acid hydrolysis (see Figure 5) indicated that these fractions contained polymers of arabinose, xylose, glucose and galactose (presumably, arabinogalactan or ISP, a xylan from cane plant and either cane starch or dextran). The electrophoretic bands from FACE analysis can be integrated. The integration results appear in Table 4. It would appear from this data that ISP is the main component of peak 2 and is, therefore, forming the polymer in peak 1 by some kind of cross-linking reaction (most likely initiated by colorant polymers). In the process of cross-linking the ratio of arabinose to galactose that can be obtained by acid hydrolysis changes.

Further evidence for the cross-linking of ISP in peak 1 can be obtained by an iodine test. Iodine forms a blue complex with starch and a red-brown complex with ISP. All peak fractions were starch negative. Although, all peaks contained arabinose and galactose only peak 2 was positive for ISP. It is likely that the arabinogalactan in peaks 1A and 1B could not form a complex with iodine because of cross-linking.

This paper reports work still in progress at SPRI to establish quantitative GPC methods that can be used to investigate the ultrafiltration processes in the sugar industry. The accomplishments to date confirm SPRI worker's theories on the fate of polysaccharides in sugarcane processing (11). Analytical GPC of membrane filtration permeates, the FACE analysis of fractions obtained from C-molasses and

nmr analysis remain to be completed. This work will be extended to process streams in raw sugar refining and beet sugar manufacture.

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Table 1. Comparative study of methods for determination of dextran in raw sugars.

Sample number	AOAC method (ppm)	Haze method (ppm)	¹ H nmr method (ppm)
1	1551	1058	1568
2	1347	953	1156
3	557	218	594
4	430	173	483
5	408	43	619
6	156	83	136

Table 2. Average degree of branching in dextrans from aqueous ethanol precipitates of raw sugars.

Ethanol Conc. (%v/v)	Serial precipitates	Parallel precipitates
50	3.8	4.0
70	5.7	4.7
85	8.3	5.1

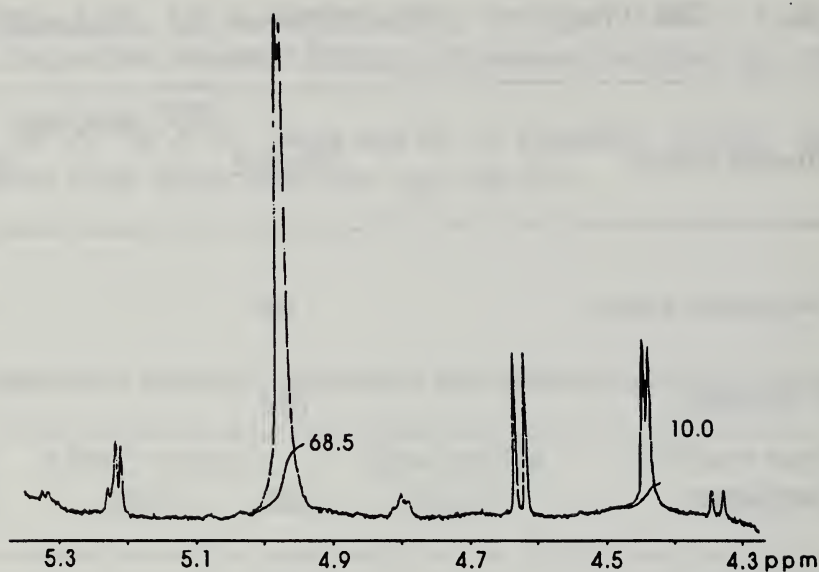
Table 3. The fate of polysaccharides in sugarcane processing.

Process stream	GPC peak area	
	Peak 1	Peak 2
Evaporator syrup	64	855
A-molasses	189	344
B-molasses	235	254
C-molasses	369	188

GPC refractive index peaks 1 and 2 are normalized to sodium concentration.

Table 4. Integration of FACE bands from gel permeation chromatography peak fractions after acid hydrolysis.

Band identity	Relative band intensity		
	Peak 1A	Peak 1B	Peak 2
Arabinose	28.6	39.4	56.0
Xylose	8.4	12.7	5.0
Glucose	46.0	28.6	1.4
Galactose	17.0	19.3	37.6



4.46 ppm, d, $J=8.30$ Hz (stractan, C1-H of β -(1-3) linked D-Galp)

4.98 ppm, d, $J=3.41$ Hz (dextran, C1-H of α -(1-6) linked D-Glcp)

Figure 1. The expanded ^1H nmr spectrum of the high molecular weight fraction of a dextran/stractan spiked raw sugar sample in D_2O at 500 MHz.

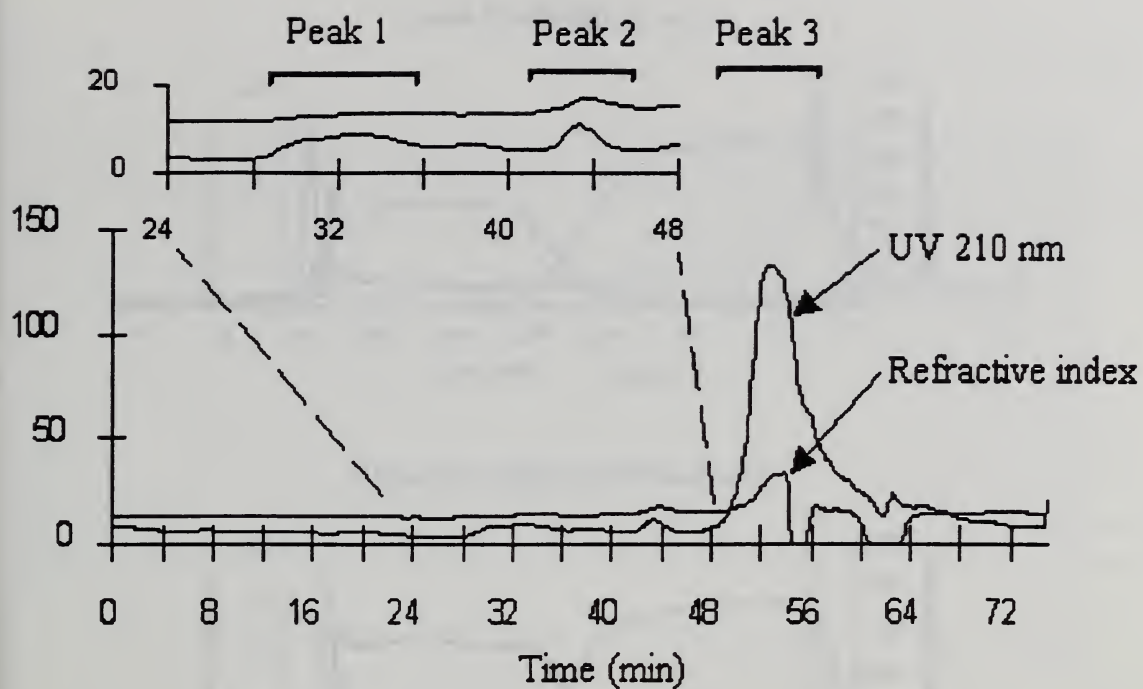
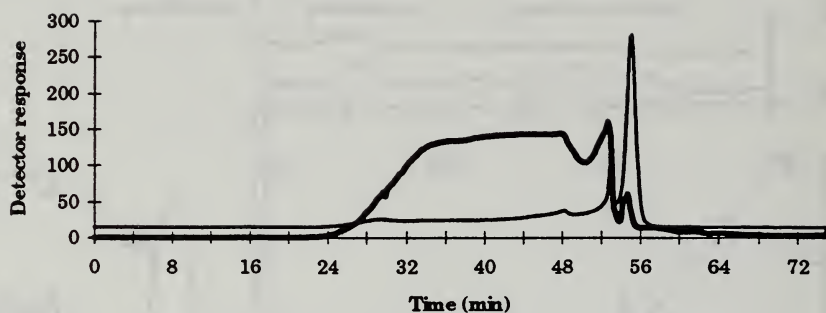
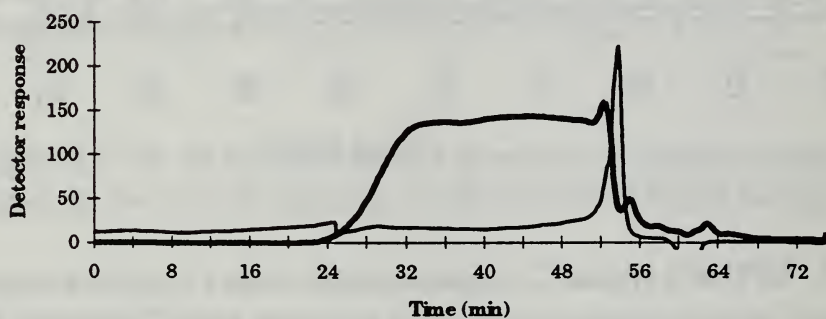


Figure 2. GPC of a typical C-molasses sample from a Louisiana sugarcane factory

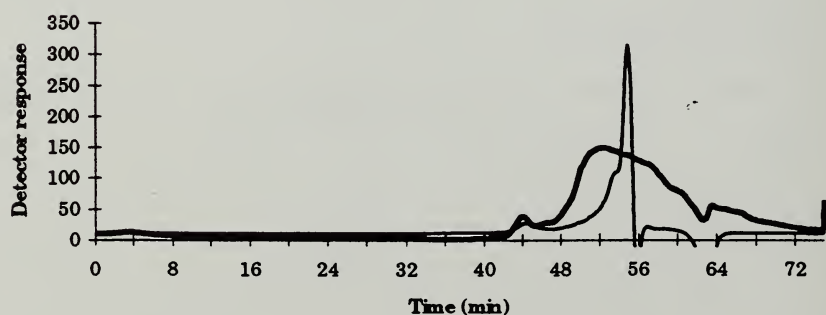
A. Elution in water



B. Elution in 10% acetonitrile



C. Elution in 0.2 M NaCl and 10% acetonitrile

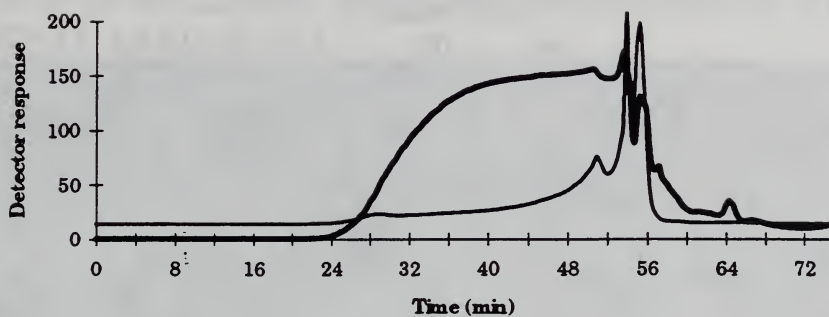


—— Refractive index detection

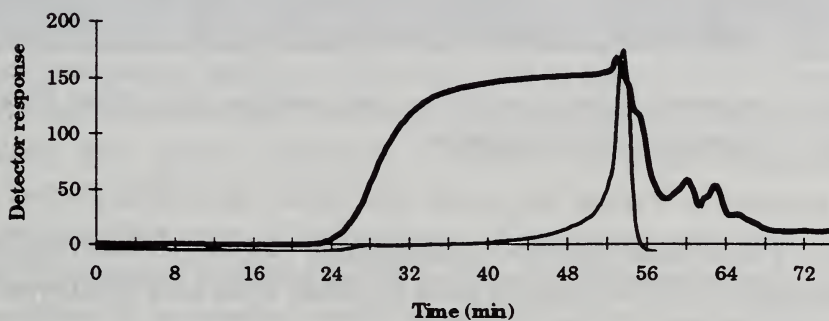
—— UV detection at 210 nm

Figure 3. Effect of eluant ionic strength on GPC of evaporator syrup ultrafiltration retentate.

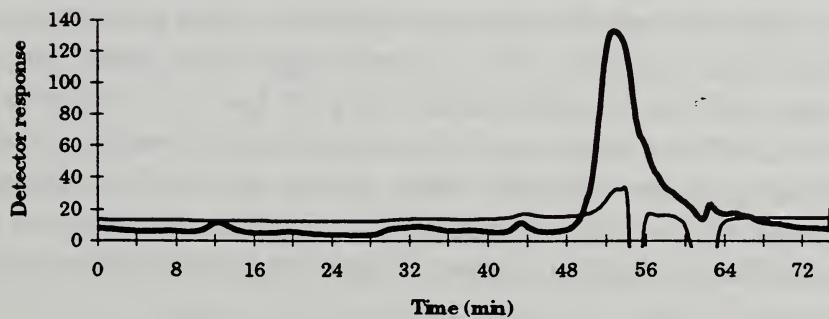
A. Elution in water



B. Elution in 10% acetonitrile



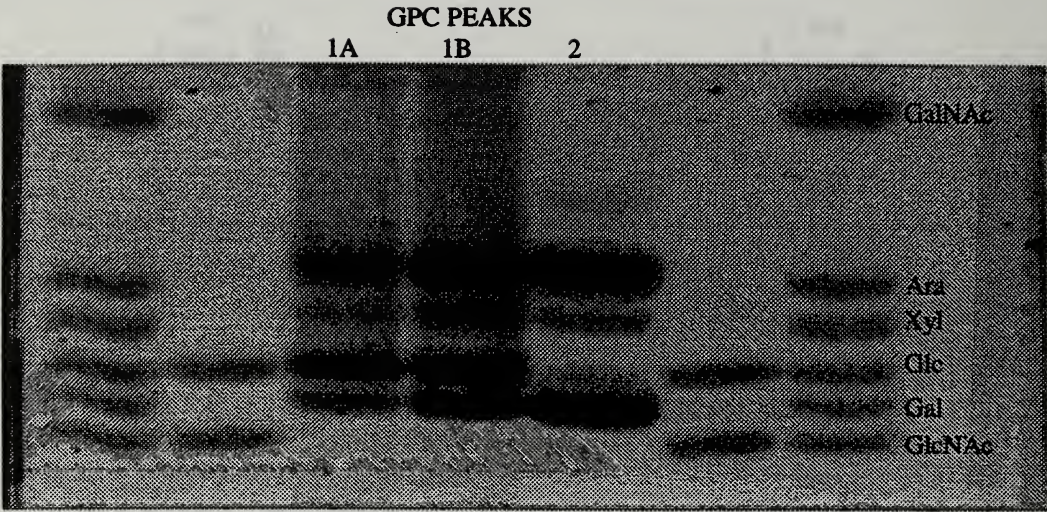
C. Elution in 0.2 M NaCl and 10% acetonitrile



— Refractive index detection

— UV detection at 210 nm

Figure 4. Effect of eluant ionic strength on GPC of C-molasses ultrafiltration retentate.



GalNAc = 2-acetamido-2-deoxy-D-galactose, Ara = arabinose, Xyl = xylose, Glc = glucose,
GlcNAc = 2-acetamido-2-deoxy-D-glucose.

Figure 5. Fluorophore-assisted carbohydrate electrophoresis (FACE) of analytical gel permeation chromatography peak fractions after acid hydrolysis.

COMPONENTS OF MOLASSES: I. SUGARCANE MOLASSES: FACTORY AND SEASONAL VARIABLES

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ABSTRACT

Goals of the current study on molasses composition include identification of polysaccharide and other components to elucidate factors affecting sugar recovery from sugarcane juice and to determine factors affecting membrane filtration processes. Nitrogen-containing and other polymers were tracked over the 1995 crushing season at a Louisiana mill, using their ratio to sucrose or other markers such as the alkali ions, sodium and potassium, halide or carboxylic acids as concentration determinants. Polymers distributed between sugar crystals and molasses were traced and those that go preferentially into crystals identified. Patterns of these compounds were established under crystallization conditions across the battery of vacuum pans and crystallization units leading to cane final molasses. Comparing evaporator syrup with "A", "B", and "C" molasses fractions by gel permeation chromatography indicated a buildup of polymers in processing, with decrease in sucrose but increase of invert products. High (900,000 to 1 million Da (Dalton)), medium (35,000 - 40,000 Da), and low molecular weight polymer (2000 to 3000 Da) containing polysaccharides, polyphenols, and nitrogen polymers such as melanoidins, increased across the four stages of the concentration and crystallization process with the low molecular weight species increasing most. Raw sugar from the "A" molasses centrifugation and washing, or combined "A" and "B", had little residue of the high and medium molecular weight polymers but still contained a large quantity of the 2000-3000 Da polymer. Variations (+/-5%) in the sucrose, invert, and purity results appear indicative of changes in processing parameters at the factory as well as in the sugarcane feed stock. Implication of nitrogenous polymers in molasses exhaustion efficiency will be discussed.

INTRODUCTION

Complex reactivity of components at the various stages of sugar juice concentration leading to crystallization during factory processing bring about both degradation of sucrose and other metabolites as well as generation of high molecular weight species

that interfere with crystallization (23). The technology of sugar crystallization is well documented, and the recent publication of the *Cane Sugar Handbook* (3) has an expanded chapter on the processes involved.

Earlier work by Gross (12) as well as Tu and coworkers (17, 18, 19) considered color and related factors in raw sugar crystals. Later Tu considered the relationship between sugar color and filterability as a polymer interference in processing (18). The properties of colorants produced by the degradation of reducing sugars in refining was investigated by Van Dam and coworkers (20) as well as J.C. Williams (22). Williams found that molecular weight, molecular size, and charge distributions of the mixture of colorants produced by model systems such as glucose and glycine under sugar refining conditions related to a wide variety of properties found in sugar refining colors and polymers.

For over 25 years the Sugar Processing Research Institute, Inc., has had an active program concerning sugarcane juice color precursors and the consequences of generation of colorant products in sugar factory operations such as crystallization or sugar quality. Papers by Carpenter (2), Clarke (6,7,8), Godshall (9,10,11), and Roberts (2,7) have explored many aspects of seasonal variability, harvest date, phenolic content, natural plant polysaccharides, microbial polysaccharides, molecular weight relationships, and acid-base indicator aspects of molasses polyaromatic condensation products. Lionnet (15) also considered the effect of some selected factors on the color in cane such as maturity, variety, and harvest conditions. In particular, Lionnet found that the phenolic content of the cane was affected by harvest date and correlated well with color. Saska and Oubrahim (16) measured molecular weight distributions of polymers in sugarcane juice, syrup, raw sugar, and molasses and characterized two fractions of higher molecular weights in the ranges of 1 million Da and 100,000 Da, respectively. The high molecular weight material, which was included in the sugar crystals, was found to be mainly dextran and the lower molecular weight material was not included in the crystals. Earlier, Tu (18) undertook work to find how high and low molecular weight colorant levels change as cane juice is processed into sugar and which colorants end up in the washed raw sugar crystal. The results indicated that the high molecular weight colorant level (>5000 Da) decreases substantially during clarification but that these colorants are still the principal ones in the sugar crystal as a result of preferential inclusion during the sugar boiling process. The low molecular weight colorants changed less during clarification and evaporation but tended to be excluded from the crystal during crystallization.

In the present study, the various molecular weight ranges of substrates for polymer formation as well as the products generated under the stress of juice evaporation will be examined. Although more emphasis is being given to conventional column chromatography for separations of low and high molecular weight, the previous paper by Edye and coworkers in this Conference has reported membrane fractionation carried out by the coworkers on these molasses fractions to concentrate polymeric by-products. Future work will also investigate sugarbeet syrup and molasses.

EXPERIMENTAL - METHODS AND MATERIALS

Evaporator syrup and molasses "A", "B", and "C" were taken as individual samples (not daily composites at the process source) for analyses described below at 12 weekly intervals from a Louisiana Sugar Factory at appropriate locations in the process stream during the period October 6 through December 27, 1995. Similar samples were taken from a Florida Sugar Factory at 8 weekly intervals from January 23 through March 12, 1996 but these samples were daily composites at the process source.

Methods were taken from the International Commission for Uniform Methods of Sugar Analysis *Methods Book*, April, 1994 (13). Analyses performed by ICUMSA methods were pol, refractometer Brix, apparent purity, pH, color, and dry substance solids by heated vacuum oven drying. High performance liquid chromatography of sucrose, glucose, and fructose for the Louisiana Factory samples was performed on a polystyrene sulfonate resin in the calcium ion form (Bio-Rad HPX-87C column, 300 x 7.8 mm, Bio-Rad, San Diego, CA, USA) and refractive index detector with Dionex AI-450 integrating computer program generally using AOAC Method #979.23 (16th Edition)(5, 21). The column was run at 80° C with 40 ppm calcium acetate in purified water (18 megohm-cm resistivity) and flow rate of 0.6 ml/min. Samples were filtered through a 0.45 µM nylon filter prior to injection. Calibration standards were run repeatedly over the entire 12 weeks of samples. Alkali metal ion analyses were performed by The Analysis Laboratory, Metairie, LA, USA. Kjeldahl amino nitrogen and Dumas (Leco) nitrogen were furnished by Woodson and Tenent Laboratory, Memphis, TN USA.

Gel permeation chromatography for sugar colorant polymers was carried out according to Khan and coworkers (14) using a Shimadzu high performance liquid chromatography system equipped with an SPD-6AV ultraviolet detector and RID-6A refractive index detector using the Shimadzu LC-10AD pump and Rheodyne 7125

injection port. A 100 μ l injection loop sample tube was employed for samples filtered through 0.2 μ l filter prior to injection. Three TSK-GEL PW-XL columns in order as G6000, G5000, and G4000 with appropriate TSK guard column, corresponding to decreasing pore sizes. Samples were uniformly diluted to 15 ° Brix prior to filtration with elution solvent. Solvent was 0.1 M sodium chloride in purified water (18 megohm-cm resistivity) that was modified with 10 % HPLC grade acetonitrile. Flow rate was 0.6 ml/min. The ultraviolet detector was set at 210 nm. Calibration standards were purchased from Scientific Polymer Products, Inc., New York, NY. These standards are sodium polystyrene sulfonates of molecular ranges from 4000 Da to 1.2 million Da. A Dionex AI-450 laboratory data analysis system was used to integrate peaks as well as to sample peak area slices for polymeric calibration curves and molecular weight determinations.

RESULTS AND DISCUSSION

Types of colorants produced from sugarcane juice during processing are listed in Table 1. Bento (5) recently reviewed these factors and applied ultraviolet spectrophotometry to study sugar colorants throughout the refining process. Van Dam and coworkers (20) studied the last point, conversion of fructose and glucose into hydroxymethylfurfural, as part of their work on monosaccharide conversion in the acidic media encountered in sugar factory operations. In the present report emphasis has been on tracking the processing streams over the entire harvest season in an attempt to determine factory as well as sugarcane parameters contributing to color formation. Separation of colorants and other polymers in Table 1 has been considered in the previous paper by Dr. Edye. In this report, an overview of parameter changes that occur in the harvest season and factory management contributing to loss of sugar recovery and deteriorating colorant formation were considered. Some of the differences between the Louisiana Factory results and the Florida Factory could be ascribed to the different methods of sampling (single point source per day in Louisiana *versus* daily composites at the process sources in Florida). For the purpose of this overview of molasses components over the crop year processing, the Louisiana and Florida data are treated separately and only compared for sake of major differences in uniformity.

Summary of Louisiana Factory Molasses Samples for the 1995 Crop

In Table 2 a summary of evaporator syrup (ES) and "A", "B", and "C" molasses (A Mol, B Mol, C Mol, respectively) analyses are listed for the Louisiana Factory

averaged over the 12 weeks 1995 crop year crushing season. Averages and standard deviations of all twelve weeks of factory operations consolidate the differences in the varieties and seasonal changes that occurred. In the following Figures and Tables variation on a weekly basis are plotted to track more closely the kinds of problems that probably occur on a daily basis. Sugars were determined by HPLC as described in the METHODS section, and results are corrected on a dry weight basis. The effect of concentration as the molasses is dehydrated and sucrose removed is apparent in the average decreases and increases across the season. These effects are more apparent in later Tables and Figures where the buildup of ash and colorant polymer is tracked.

The following several Figures illustrate how Louisiana Factory operation changes with seasonal variation or varietal quality of sugarcane during the period. Figure 1 illustrates this seasonal variation of molasses Brix over the 12 weeks of Louisiana Factory operation. This closely follows in Figure 2 the seasonal variation of the more accurate measurement of molasses dry solids over the same 12 weeks. The plot of Brix and dry solids in Figure 3 demonstrates good correspondence of both measurements from the Louisiana Factory samples.

Gel permeation chromatography of colored polymers generated in the Louisiana Factory sugar crystallization processes are shown in Table 3. Ratios of ultraviolet absorbing molecules over the molecular weight range are presented to illustrate the directions of formation of high molecular weight polymer (Peak 1) from low molecular weight material (Peak 3) through the intermediacy of medium molecular weight colorant (Peak 2). The ratio of low molecular weight colored material to high molecular weight final product is quite high (800-900 to 1). Interestingly, the "A" sugar contains little of the higher molecular weight fractions (Peaks 1 and 2) but mostly all Peak 3 as a contaminant of the Factory product.

Summary of Florida Factory Molasses Samples for the 1996 Crop

In Table 4 analyses of evaporator syrup and molasses fractions for the Florida Factory, 1996 Crop Year, are presented. Averages and standard deviations of all eight weeks summarize for the Florida Factory the trends shown in Table 2 for the Louisiana samples. The seasonal variation of molasses Brix over 8 weeks of Florida Factory operations are plotted in Figure 4. These Brix concentrations follow well data presented in Figure 5 for seasonal variation of molasses dry solids over 8 weeks of Florida Factory operation. The variability on a week to week basis is less in the Florida samples than the Louisiana.

Relationship of Sugar Analyses with Colorant Formation

The plot of apparent purity (Pol/ Brix) of sugar in Figure 6 from the Louisiana Factory operation demonstrates the exhaustion of sucrose from the molasses throughout the season. The wide variability in the Final C Molasses and the trend towards much higher sucrose content near the end of the season could be related to harvest date and maturation factors or an increase in the amount of cane processed at the factory (See also footnote below). Some weather related changes could be ascribed to frosts which occurred in the 12/2 through 12/27 weeks. Damage to the cane stalks will facilitate microbial growth, and microbially produced polymers such as dextran will also impede crystallization. Similarly, sucrose purity is plotted in Figure 7 using HPLC values for sucrose from Table 5 and dry weight solids. As a seasonal variation in the Louisiana Factory operation, the latter weeks show a trend to less molasses exhaustion of sucrose as pointed out above. As shown in Figures 8 and 9, however, fructose and glucose do not follow any significant trends across the season except that there is always more fructose than glucose as is to be expected at the acidic pH of factory operations. In Table 6 and Figure 10 invert sugar increases regularly over the four factory stages but except for weekly rises and falls, does not show the greater seasonal accumulation in the later half of the season shown by sucrose. The apparent purity of the Florida Factory sugar presented in Figure 11 is seen to be much more regular than that of the Louisiana Factory. This may be indicative of more mature cane, a more stable growing season in Florida as well as other factors such as mixture of varieties and gradual chilling of the air toward the end of the Louisiana growing season¹.

Sugar Colorant and pH Variation in Louisiana and Florida Factory Samples

The ICU color analysis is pH dependent because the polymers are themselves acid-base indicators whose absorbencies are sensitive to pH (4). This is amply illustrated in Figures 12, 13, and 14 for Louisiana Factory samples measured at pH's of 4, 7, and 9. In Figure 15 a plot of the averaged values for ICU color presented earlier in Table 2 are plotted at the three pH's to delineate these acid-base effects on the ultraviolet-visible light absorption of the polymers. In Figure 16 the ICU color at

1

¹It should be noted that the Louisiana factory was operating with new crystallizing equipment, which did not function correctly and gave poor exhaustion for most of the crop.

pH 7 for the 8 weeks of the Florida Factory season are compared. With the exception of the week of 2/19 the color values of the Florida Factory are somewhat lower than those of the Louisiana Factory across the whole processing system of evaporator syrup, "A" molasses, "B" molasses, and "C" molasses. Why less colorant is being generated in the Florida samples is not immediately apparent. When the invert levels in Florida samples become available, one more contributing factor may be evident. Also the mature Florida cane may release less phenolic color into the juice than the immature Louisiana cane to make another contributing factor.

Natural pH across the process as well as the season is plotted in Figure 17 for the Louisiana Factory. The pH tends to become higher as the season progresses in the Louisiana samples except in the "C" molasses. This is controlled, of course, by adjustment at the flocculation step. Compared to the Florida Factory plotted in Figure 18, the Louisiana Factory is averaging about a pH unit higher than Florida but the Florida Factory is running more regularly in the later part of the season. These pH differences are very real and important since at the lower pH more sucrose can be hydrolyzed and generate monosaccharides which can degrade and polymerize to form the damaging colorant polymers. In Figure 19 a bar graph of the overall averages and standard deviations of the Florida and Louisiana Factory pH profiles is given. The Florida Factory has a tighter standard deviation for each stage of the processing but an overall steady low pH compared to the evaporator syrup, "A" molasses, and "B" molasses from the Louisiana Factory. However, the pH of the "C" molasses from Louisiana is somewhat lower than that of the Final C Molasses from Florida, perhaps because of a higher level of organic acid in the immature cane.

Alkali Metal Ion (Sodium and Potassium) Content of Louisiana Factory Molasses as a Variable in Processing

The sodium and potassium ions of sugarcane syrup and molasses are in a ratio of 1 to 90 or 100. As a rich source of potassium, molasses should be considered a "Heart-healthy food". As molasses exhaustion of sucrose takes place and polymers build up in the Final "C" Molasses, a concurrent higher concentration of ash also is building up. This ash is made of salts that can upon hydrolysis be either basic or acidic. As such these salts can also catalyze degradation reactions of sugars as well as promote sugar colorant polymerizations. In Table 7 representative sodium and potassium atomic absorption results are presented for the Louisiana Factory showing the concentration changes across processing stages. These values are plotted as bar graphs in Figures 20 and 21.

To illustrate the relationship of sodium and potassium to reactivity in sugar degradation and colorant formation, in Figure 22 a regression analysis is plotted for sodium and potassium concentrations, respectively, *versus* % invert sugar for the Louisiana Factory. A linear relationship results with $R^2 > 0.9$ for each ion as it regressed *versus* % invert sugar in evaporator syrup, "A", "B", and "C" molasses. The significance of this linear relationship between % invert and ash components is emphasized by the fact that the same sodium and potassium regression against ICU color at three pH's is also linear, illustrating the role of ash in color formation (graphs not shown).

Kjeldahl Amino Nitrogen and Dumas Total Nitrogen in the Louisiana Factory Processing Stages

Relative Amounts of Nitrogen in "A" Raw Sugar

Dumas nitrogen analyses of Louisiana Factory molasses representative of the 1995 Crop operation are shown in Figure 23. In Figure 24 are compared Kjeldahl amino nitrogen analyses of Louisiana Factory molasses representative of the 1995 Crop operation. The Dumas nitrogen analysis is lower than the Kjeldahl overall. It is difficult to explain these differences except to say that perhaps the Kjeldahl is high because titratable anions are being co-distilled giving a higher acid titration value or electrode response. In any case, the nitrogen concentration also builds across the molasses crystallization process, and like the alkali metal ions is a stable marker upon linear regression with other parameters, as have been discussed above. In the "A" raw sugar samples in Figures 23 and 24, the nitrogen is much lower than that of the "A" molasses, indicating that the nitrogenous materials are not readily co-crystallized with the sugar product.

Sugar Colorant and Polymer Distributions During Processing

In Figure 25 gel permeation chromatograms of Louisiana Factory molasses are presented in an overlay to show separation of various molecular weight ranges. As discussed earlier, integration of these chromatograms are the source of information averaged over the season in Table 3 for each of the sugar crystallization processing stages. The point is made again in the following plots of gel permeation chromatograms that over the entire season, rather regular patterns emerge of high, medium, and low molecular weight polymers as temperature, concentration, and removal of sugars progress. Figure 26 shows the seasonal variation of Louisiana

Factory molasses by gel permeation chromatography, demonstrating generation of polymers with high molecular weight (900,000 Da.). It is apparent that this polymer is of greater concentration for all the stages than the intermediate molecular weight polymer (38,000 Da) in Figure 27. On the other hand, the lowest molecular weight polymeric species (2600 Da) continues to increase across the stages as well as during the season. The 2600 Da molecular weight range also predominates in raw sugar (data not presented) as it is carried over during crystallization as an inclusion product.

Summary with Future Directions of the Research

Examination of various molecular weight ranges of substrates for polymer formation and the products generated by juice evaporation has led to a model of anticipated changes occurring during cane sugar processing. Chromatography for separations of low and high molecular weight components permits monitoring these variables and better control the process. Definition of components and properties at various stages of crystallization for different locations during the harvest season gives insight into the dynamics of the system. In future work, membrane fractionation of these polymeric by-products will be carried out to improve characterization of the products. Investigation of sugarbeet syrup and molasses from different locations, varieties, and factories will extend knowledge of this system. Ion chromatography of carboxylic acids, inorganic anions, and mineral cations will be investigated to correlate with product characterization at different locations during the harvest season.

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23. Zerban, F. W. (1947). The color problem in sucrose manufacture. *Technical Report No. 2. Sugar Research Foundation, Inc. New York.* pp. 1 - 31.

Table 1. Types of colorants produced during sugarcane and sugarbeet juice processing.

- ▶ 1. Phenolics and flavonoids which react under heat and free radical conditions to produce modified aromatic polymers.
- ▶ 2. Carbonyl-amine reactants form many kinds of Schiff base intermediates, which under intense heat and concentration polymerize to form melanins and melanoidans in Maillard chemistry.
- ▶ 3. Pyrolytic and other thermal degradation products of reactive intermediates, which then accumulate or further modify polysaccharides, phenolics, or the polymers described in 1. and 2.
- ▶ 4. Alkaline degradation products of fructose (ADF), which are highly reactive hydroxycarbonyls, that self-polymerize in their own patterns.

Table 2. Summary of evaporator syrup and molasses analyses. Louisiana factory, 1995 crop year. Averages and standard deviations of all twelve weeks of factory operation. Sugars determined by HPLC and corrected on dry weight basis.

Table 2. Summary of Evaporator Syrup and Molasses Analyses. Louisiana Factory, 1995 Crop Year. Averages and Standard Deviations of All Twelve Weeks of Factory Operation. Sugars Determined by HPLC and Corrected on Dry Weight Basis.								
SAMPLE	Brix Degrees	Std. Dev. ±	Polarity Degrees	Std. Dev. ±	Apparent Purity % w/wt	Std. Dev. ±	Process pH	Std. Dev. ±
Evaporator Syrup	66.3	2.09	54.76	1.80	82.60	2.44	7.02	0.26
A Molasses	75.24	3.85	54.22	3.32	72.17	3.72	6.63	0.19
B Molasses	77.09	3.40	49.21	2.40	63.86	2.55	6.62	0.19
C Molasses	82.64	2.13	38.51	2.27	46.63	3.15	5.98	0.19
SAMPLE	Average % Solids	Std. Dev. ±	ICU COLOR pH4	Std. Dev. ±	ICU COLOR pH 7	Std. Dev. ±	ICU COLOR pH 9	Std. Dev. ±
Evaporator Syrup	65.44	2.29	20658	2764	23018	6896	47547	14056
A Molasses	71.64	5.44	34005	8249	73288	28052	141600	50533
B Molasses	76.77	4.25	43585	14507	84430	25972	166585	46848
C Molasses	79.96	1.78	106868	27334	157946	52011	340486	252668
SAMPLE	Sucrose %w/wt	Std. Dev. ±	Glucose %w/wt	Std. Dev. ±	Fructose %w/wt	Std. Dev. ±	Invert Sugar %Glc + Fru	Std. Dev. ±
Evaporator Syrup	79.27	11.79	1.36	0.30	1.48	0.76	2.83	0.97
A Molasses	65.15	11.62	3.01	0.69	3.81	0.43	6.82	1.01
B Molasses	58.61	9.39	3.19	0.63	4.35	0.88	7.54	0.96
C Molasses	44.59	10.49	3.38	1.16	6.82	1.67	10.20	2.16

Table 3. Gel permeation chromatography of polymers generated in sugar crystallization process. Louisiana factory, 1995 crop year. Ratios of ultraviolet absorbing molecules over the molecular weight range.

PEAK 1 900,000 Da					
Week	E.S.	A Mol	B Mol	C Mol	
10-6	0.31	1.00	1.39	2.17	
10-11	1.31	3.65	3.18	7.45	
10-19	1.45	3.63	3.90	3.94	
10-27	1.00	3.68	2.91	7.22	
11-03	1.11	3.30	5.57	7.53	
Average	1.04	3.05	3.39	5.66	
Std. dev.±	0.39	1.03	1.37	2.20	

Week	E.S.	A Mol	B Mol	C Mol	
10-6	0.19	0.63	0.61	1.25	
10-11	1.20	3.37	2.45	5.05	
10-19	0.97	2.78	3.58	3.80	
10-27	0.81	2.51	2.82	4.06	
11-03	0.73	2.48	3.63	4.80	
Average	0.78	2.35	2.62	3.87	
Std. dev.±	0.33	0.92	1.10	1.40	

Week	E.S.	A Mol	B Mol	C Mol	
10-6	65	185	240	450	
10-11	361	599	561	817	
10-19	365	599	612	753	
10-27	267	532	579	840	
11-03	268	550	563	807	
Average	277	495	625	734	
Std. dev.±	116	157	139	145	

Table 4. Analyses of evaporator syrup and molasses fractions. Florida factory, 1996 crop year. Averages of all eight weeks with standard deviations.

SAMPLE	Brix Degrees	Std. Dev. ±	Polarity Degrees	Std. Dev. ±	Apparent Purity % w/wt	Std. Dev. ±
Evaporator Syrup	65.1	0.88	54.34	0.64	83.5	1.24
A Molasses	70.3	1.46	47.85	1.56	68.1	1.89
B Molasses	69.9	1.11	42.83	1.70	61.3	1.97
C Molasses	79.8	2.00	33.84	1.66	42.4	1.47

SAMPLE	Average % Moisture	Std. Dev. ±	Average % Solids	Std. Dev. ±	Process pH	Std. Dev. ±
Evaporator Syrup	36.04	0.50	63.96	0.50	6.43	0.20
A Molasses	31.54	1.85	68.46	1.85	6.37	0.08
B Molasses	31.77	0.99	68.23	0.99	6.35	0.09
C Molasses	23.57	2.48	76.43	2.48	6.09	0.05

SAMPLE	ICU COLOR pH4	Std. Dev. ±	ICU COLOR pH 7	Std. Dev. ±	ICU COLOR pH 9	Std. Dev. ±
Evaporator Syrup	9194	3274	18613	6545	48055	17401
A Molasses	29756	7080	52804	13971	123854	39365
B Molasses	38431	13321	66602	23608	157521	57238
C Molasses	63836	22391	105848	32483	225969	72888

Table 5. High performance liquid chromatography of sucrose. Dry weight basis. % purity Louisiana factory, 1995 crop year.

Date	E.S %	Mol. A %	Mol. B %	Mol. C. %
10-6	64.65	54.80	52.19	38.11
10-11	64.97	47.88	56.88	22.82
10-19	64.34	54.89	44.74	37.60
10-27	65.21	56.17	51.94	38.82
11-3	69.30	60.04	51.73	42.87
11-10	83.47	54.89	49.25	36.13
11-17	85.95	61.66	54.83	47.94
11-21	89.05	78.85	62.43	59.24
12-2	88.03	78.50	72.95	44.87
12-8	90.75	77.05	72.91	50.49
12-19	92.64	75.65	60.79	54.83
12-27	92.88	81.47	72.70	61.41
AVERAGE	79.27	65.15	58.61	44.59
STD DEV	11.79	11.62	9.39	10.49

Table 6. High performance liquid chromatography of invert sugar (glucose plus fructose). Dry weight basis. Louisiana factory, 1995 crop year.

Date	E.S. %	A Mol %	B Mol %	C Mol %
10\6	2.42	5.56	7.62	10.85
10\11	2.78	9.22	4.83	5.30
10\19	2.80	6.30	8.63	12.35
10\27	1.92	7.77	7.65	11.31
11\3	1.55	5.89	7.33	9.84
11\10	5.57	6.93	7.68	7.24
11\17	3.26	7.42	8.47	14.02
11\21	2.82	7.18	7.64	10.80
12\2	2.49	6.53	7.74	10.65
12\8	2.03	5.37	6.98	8.93
12\19	3.34	7.04	7.43	10.60
12\27	3.06	6.58	8.50	10.51
AVERAGE	2.84	6.82	7.54	10.20
STD. DEV.	0.97	1.01	0.95	2.16

Table 7. Sodium and potassium atomic adsorption results. Louisiana factory, 1995 crop year.

Week	Sodium (ppm)				Potassium (ppm)			
	E.S.	A Mol	B Mol	C Mol	E.S.	A Mol	B Mol	C Mol
10-11	228	348	479	822	20768	18460	25392	43636
10-27	205	397	564	419	13330	27000	33165	27022
12-8	138	378	646	823	12804	24037	33396	42480
Average	190	374	563	688	15634	23165	30651	37712
Std. dev.	38	20	68	190	3636	3541	3720	7574

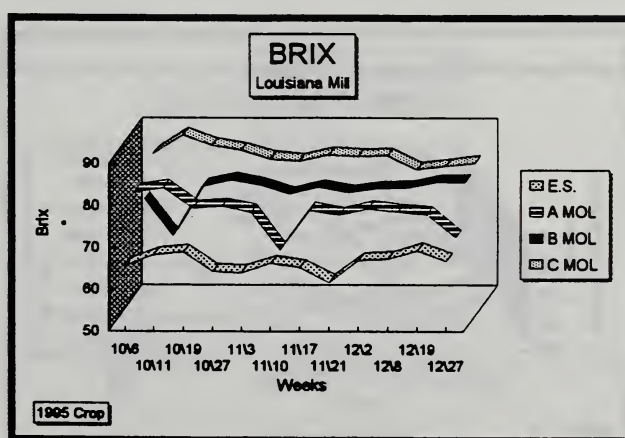


Figure 1. Seasonal variation of molasses Brix over 12 weeks of Louisiana factory operation, 1995 crop year.

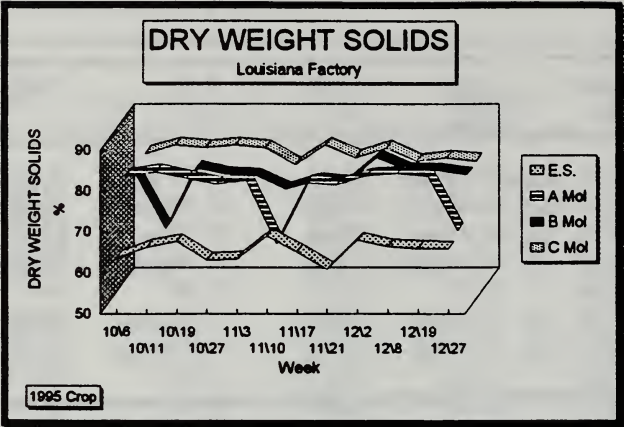


Figure 2. Seasonal variation of molasses dry solids over 12 weeks of Louisiana factory operation, 1995 crop year.

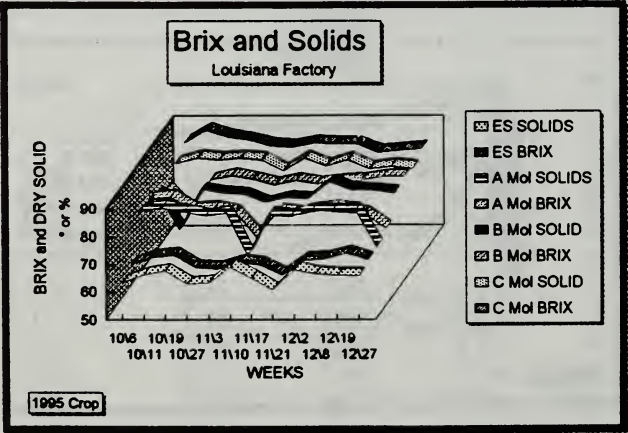


Figure 3. Plot of Brix and dry solids to demonstrate correspondence of both measurements from Louisiana factory operation, 1995 crop year.

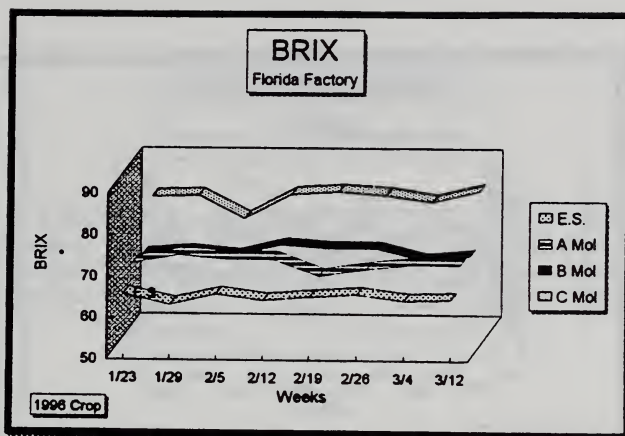


Figure 4. Seasonal variation of molasses Brix over 8 weeks of Florida factory operation, 1996 crop year.

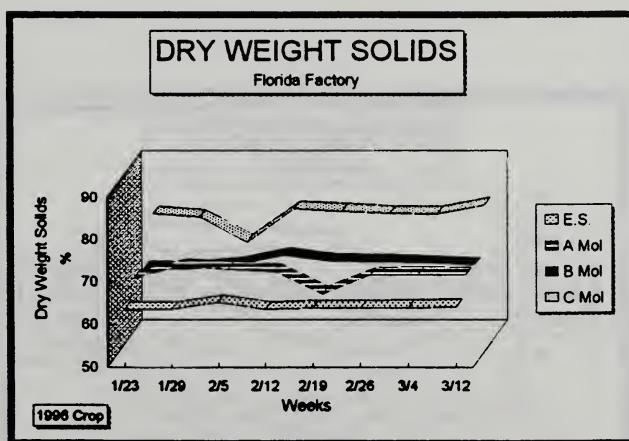


Figure 5. Seasonal variation of molasses dry solids over 8 weeks of Florida factory operation, 1996 crop year.

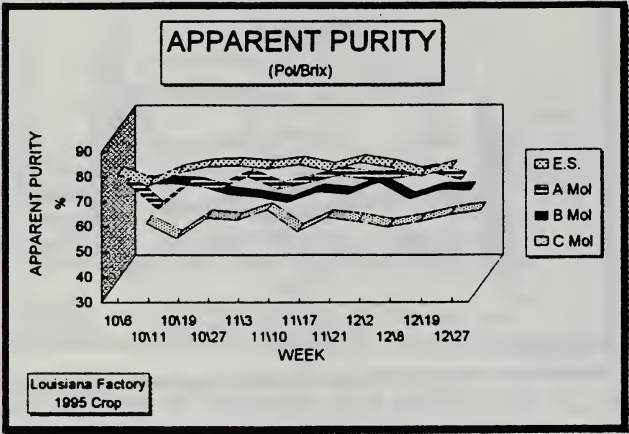


Figure 6. Plot of apparent purity (pol/Brix) of sugar from Louisiana factory operation, 1995 crop year.

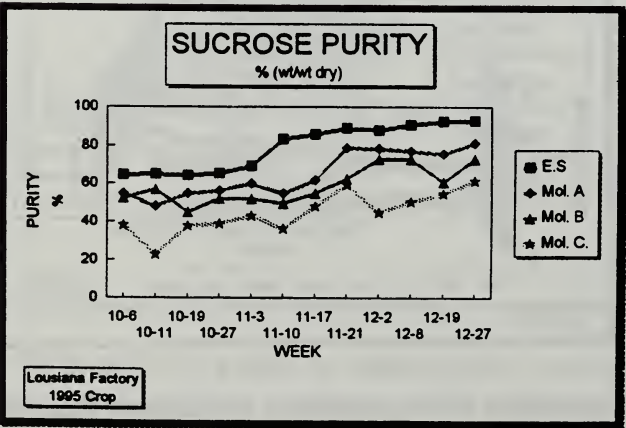


Figure 7. Plot of sucrose purity (sucrose by HPLC/dry weight solids) from Louisiana factory operation, 1995 crop year.

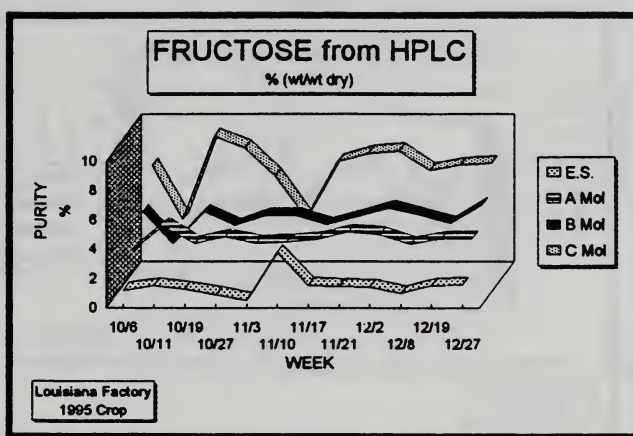


Figure 8. Plot of fructose (fructose by HPLC/dry weight solids) from Louisiana factory operation, 1995 crop year.

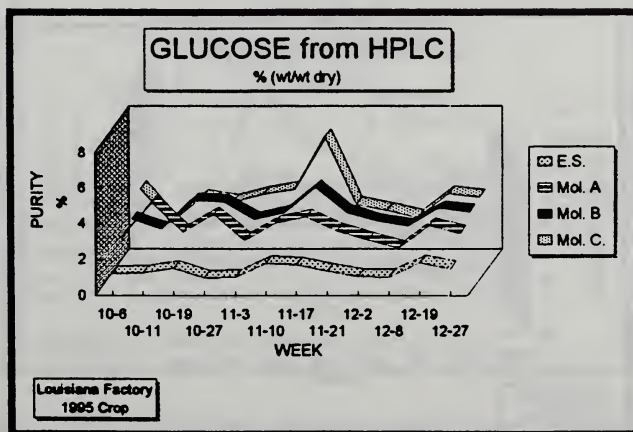


Figure 9. Plot of glucose (glucose by HPLC/dry weight solids) from Louisiana factory operation, 1995 crop year.

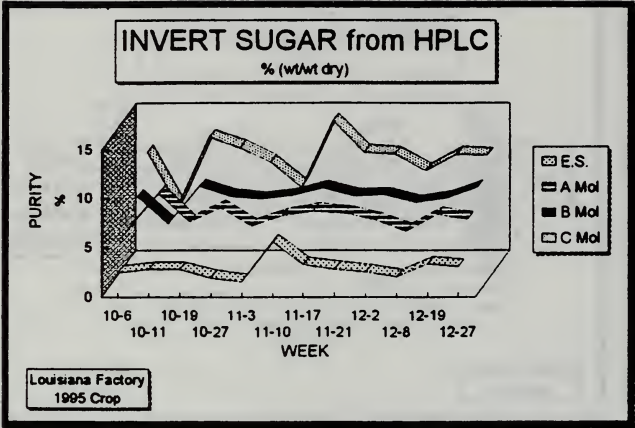


Figure 10. Plot of invert sugar (fructose plus glucose by HPLC/dry weight solids) from Louisiana factory operation, 1995 crop year.

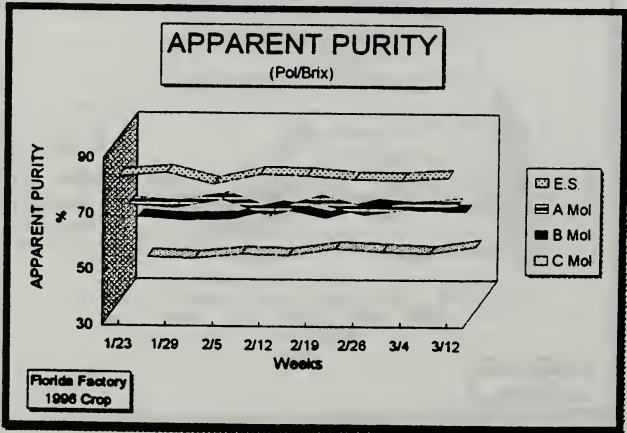


Figure 11. Plot of apparent purity (pol/Brix) of sugar from Florida factory operation, 1995 crop year.

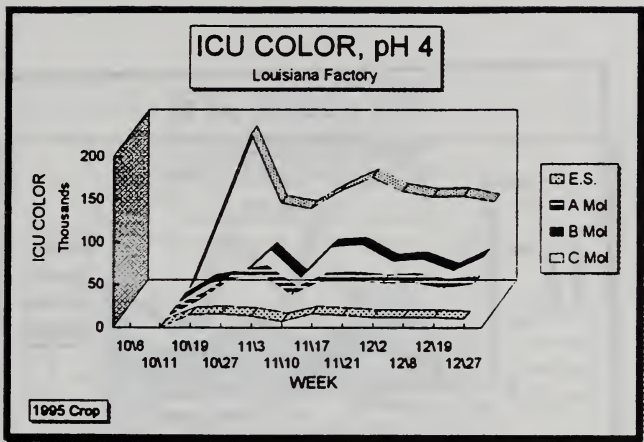


Figure 12. ICU color, pH 4, Louisiana factory operation, plotted for 1995 crop year.

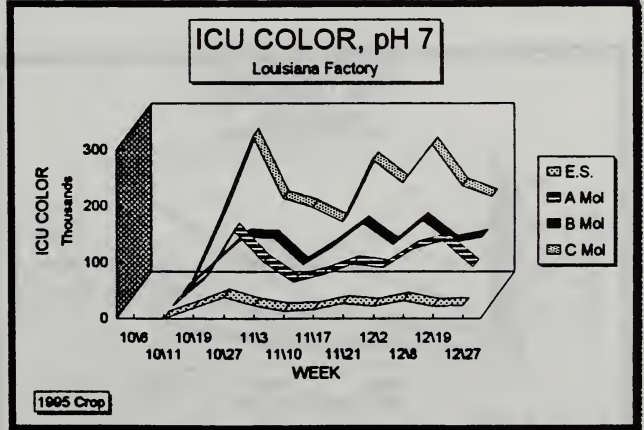


Figure 13. ICU color, pH 7, Louisiana factory operation, plotted for 1995 crop year.

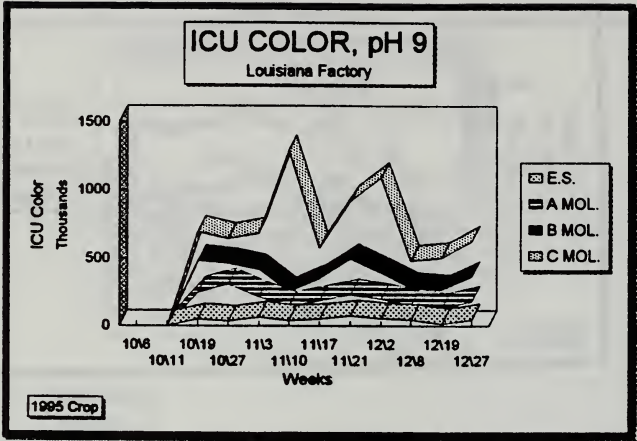


Figure 14. ICU color, pH 9, Louisiana factory operation, plotted for 1995 crop year.

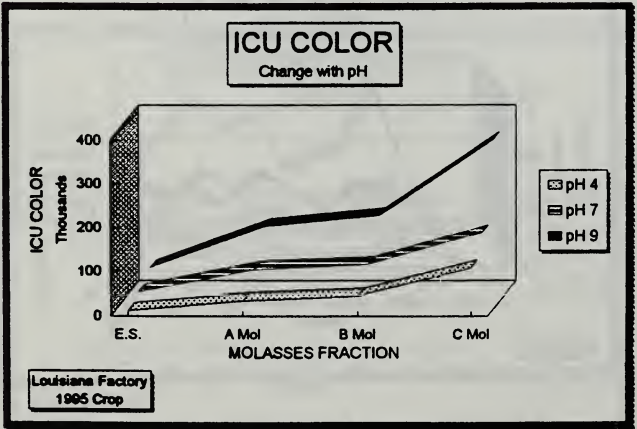


Figure 15. Composite plot of color values for Louisiana factory operation, 1995 crop year, at three pH values to demonstrate indicator effect across the range.

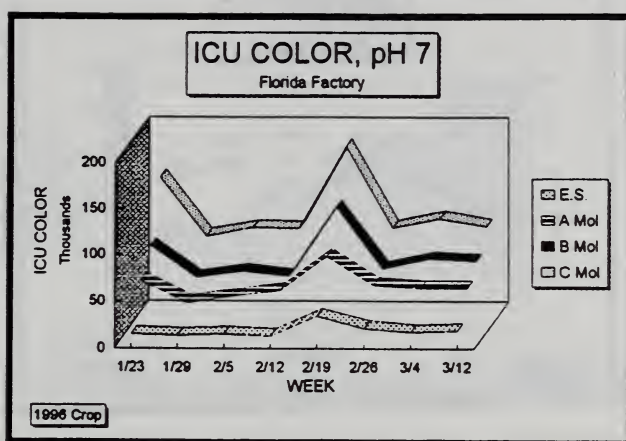


Figure 16. ICU color, pH 7, Florida factory operation, plotted for 1996 crop year.

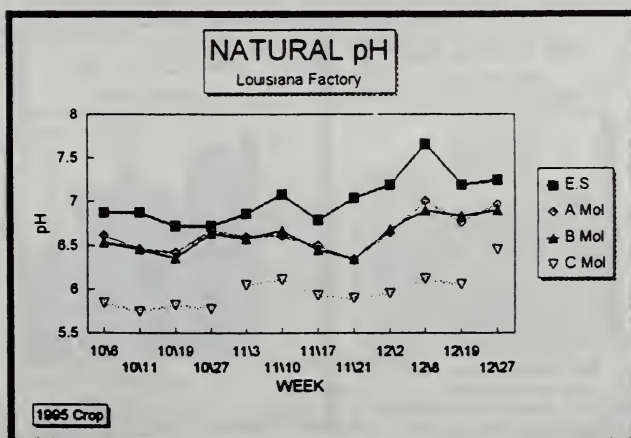


Figure 17. Natural pH of factory molasses, Louisiana factory operation, compared over the 1995 crop year.

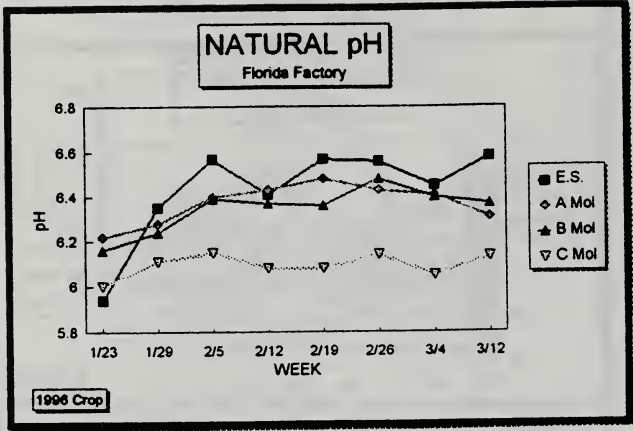


Figure 18. Natural pH of factory molasses, Florida factory operation, compared over the 1996 crop year.

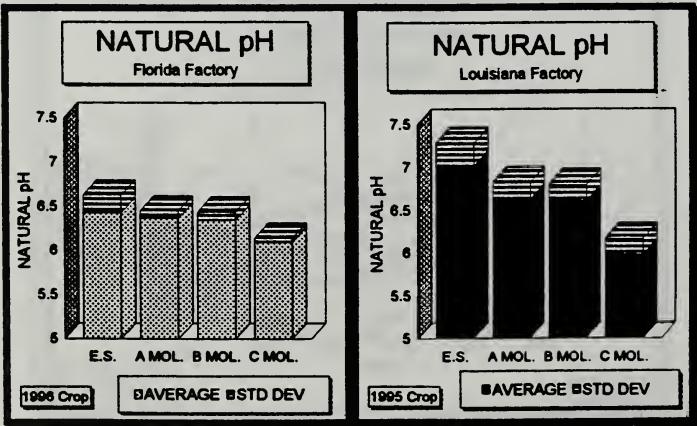


Figure 19. Comparison of pH profiles for Louisiana 1995 and Florida 1996 factory operations.

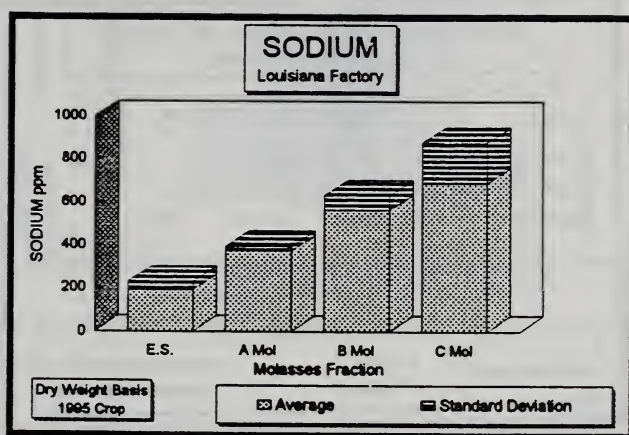


Figure 20. Sodium analyses of Louisiana factory molasses representative of the 1995 crop operation.

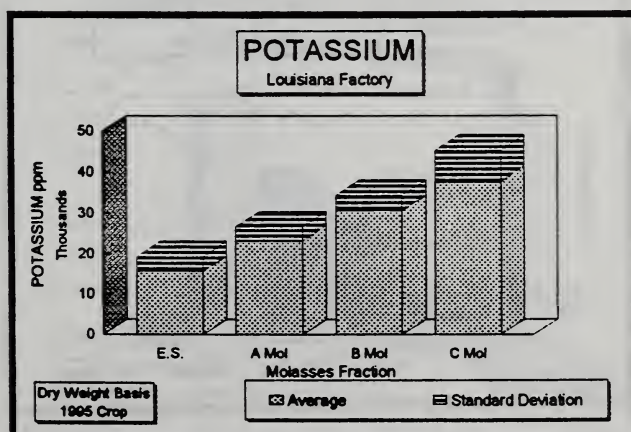


Figure 21. Potassium analyses of Louisiana factory molasses representative of the 1995 crop operation.

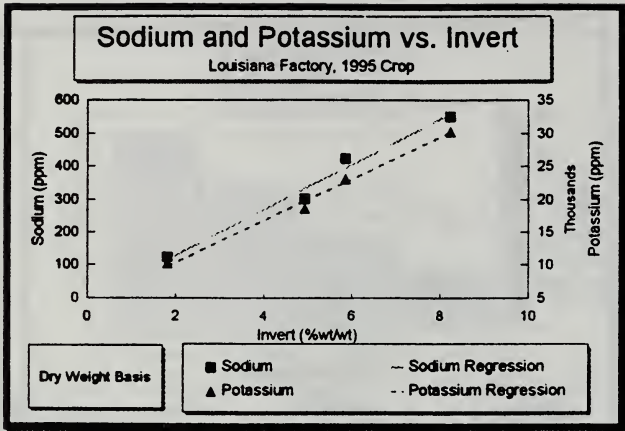


Figure 22. Sodium and potassium, Louisiana factory plotted with invert concentration - linear regression.

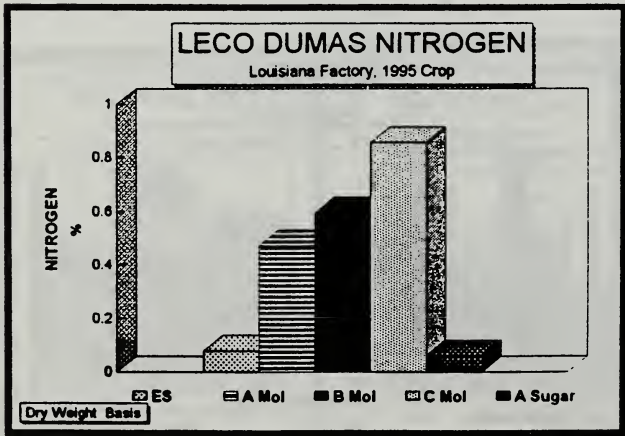


Figure 23. Dumas nitrogen analyses of Louisiana factory molasses representative of the 1995 crop operation.

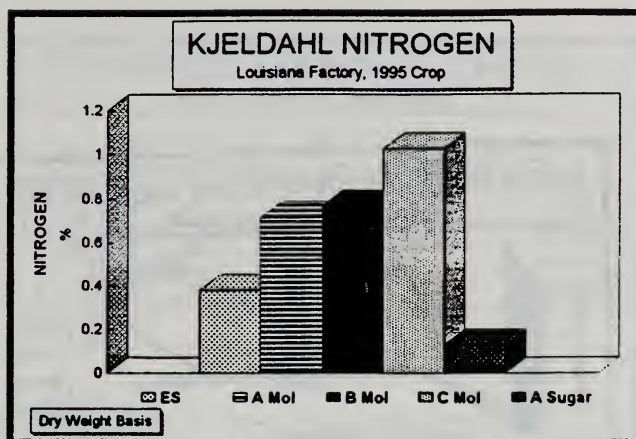


Figure 24. Kjeldahl nitrogen analyses of Louisiana factory molasses representative of the 1995 crop operation.

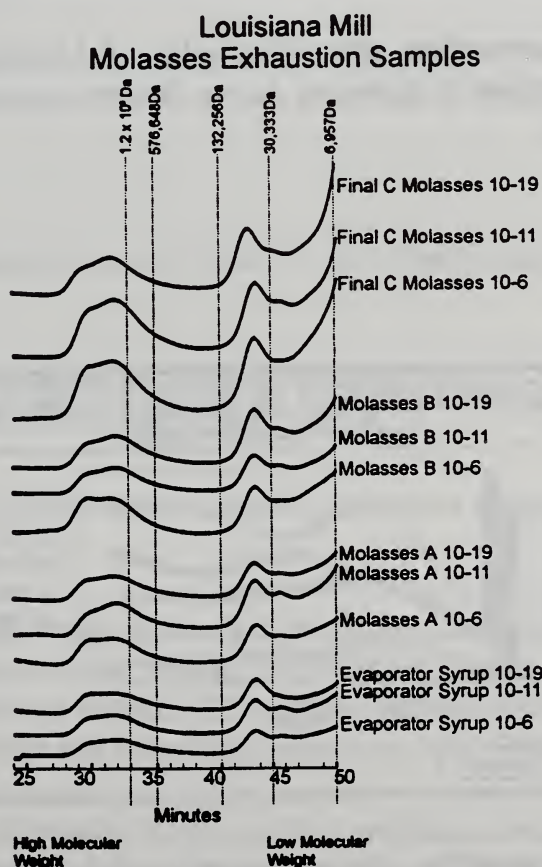


Figure 25. Gel permeation chromatograms of Louisiana factory molasses from 1995 crop year showing separation of various molecular weight ranges.

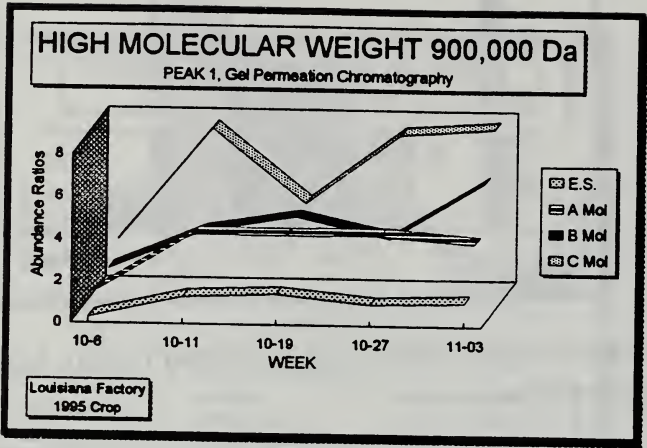


Figure 26. Gel permeation chromatography of Louisiana factory molasses demonstrating generation of polymers during factory operation. High molecular weight - 900,000 Da.

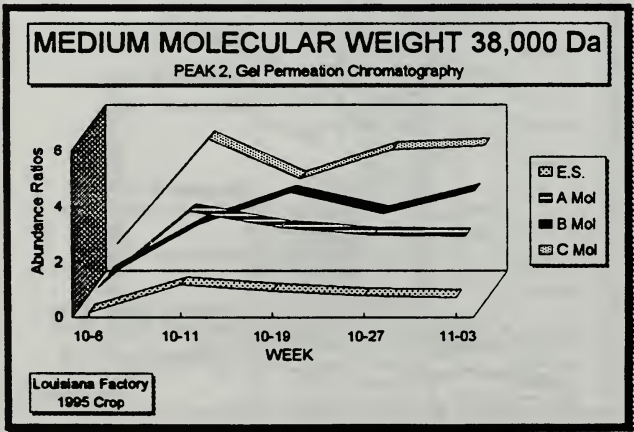


Figure 27. Gel permeation chromatography of Louisiana factory molasses demonstrating generation of polymers during factory operation. High molecular weight - 38,000 Da.

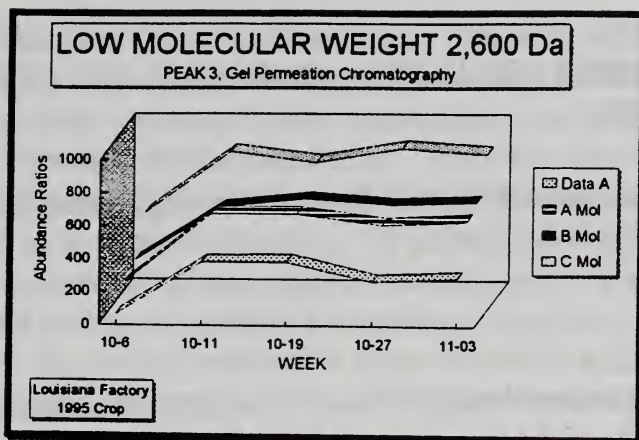


Figure 28. Gel permeation chromatography of Louisiana factory molasses demonstrating generation of polymers during factory operation. High molecular weight - 2,600 Da.

DISCUSSION

Question: One property we would like to see is the viscosity - do you have these numbers?

Vercellotti: No, we have not yet obtained viscosity numbers to incorporate into our model. There are gaps that we still have to fill in.

Question: I am surprised at the variation in sucrose and purity: for week 2, the figures are very low.

Vercellotti: That sample was obviously taken during a wash cycle. Other variations reflect changes in cane variety, rainfall, and weather, including 3 frosts: the factory was working to maximum capacity; there were problems with new crystallizers and molasses was sometimes not completely exhausted.

Comment: This information is also useful to refineries because, as cane factories know, problems in raw sugar production are passed on to refineries. The more information we develop on raw sugar production, the better the situation is for everyone.

CANE AND BEET QUALITY ANALYSES BY NEAR INFRARED SPECTROSCOPY

Les A. Edye and Margaret A. Clarke, Sugar Processing Research Institute, Inc., New Orleans, Louisiana, USA

ABSTRACT

This presentation examines the use of near infrared (NIR) spectroscopic methods for the analysis of quality of the primary agricultural products of the sugar industry (i.e., sugarcane and sugarbeets). The Louisiana sugarcane payment system is based on weight and on quality analysis of core samples from cane hauling bins. The North American beet sugar industry also incorporates quality analyses in grower payment systems and beet variety development programs. The conventional quality parameter of the Louisiana sugar industry, theoretical recoverable sugar (TRS), is calculated from the results of several analyses of core samples (viz., pressed juice sediment, refractometric dissolved solids and polarimetric sucrose, and cane moisture). In this study NIR spectroscopy has been utilized for direct prediction of TRS. Conventional analysis and NIR spectroscopic analysis of core samples for the last two sugarcane harvests are compared. The effect of cane preparation methods and sample presentation on NIR calibration development will be discussed. Similarly, conventional analyses and NIR analysis of beet quality for the last three beet harvests are compared.

INTRODUCTION

NIR multicomponent analyses of forage, fiber, grain and cereal are well documented (1,2). The possibility for application of NIR spectroscopy to cane and beet quality assessment (for payment systems and for variety development) has prompted several feasibility studies.

Cane quality assessment by NIR spectroscopy

The feasibility studies on cane quality assessment by NIR spectroscopy include both reflectance spectroscopy of finely chopped cane and transmission spectroscopy of expressed juice. Berding, et al. (3-6), have concentrated on cane quality assessment for variety development using hand cleaned and finely chopped cane; the conventional

analyses were performed in their own laboratory. The early studies (3,4) utilized a 19 filter instrument for the analysis of expressed juice and fibrated (finely chopped) cane. The results from expressed juice approached the quality (accuracy and precision) required for variety development trials. While the results from the analysis of fibrated cane were insufficiently accurate, the substantial advantages of direct analysis of fibrated cane were encouraging. In subsequent studies (5,6) the use of scanning NIR instruments and a large cassette module sample presentation (80 x 80 x 1,000 mm) effected an improvement in the quality of analytical results to the point where Berding, et al. (6), recently reported a move from NIR R&D to routine at-line NIR analysis of fibrated cane samples in the variety development program.

Clarke and Edye (7-9) have concentrated on cane quality assessment for grower payment using cane samples and conventional analyses directly from a Louisiana core lab. Recently, the Sugar Milling Research Institute in South Africa (10) has also taken an interest in NIR analysis (reflectance spectroscopy) of finely chopped cane for the prediction of DAC (Direct Analysis of Cane: pol, Brix and moisture: for a cane payment system); their initial study on less than 200 samples without validation was encouraging and further investigation of this NIR application will be reported at the 1996 South African Sugar Technologists' Association meeting. In the 1994-95 season the factory at Vale Do Rosario, Brazil ran NIR parallel to core sample analysis systems (standard press method and hot water extraction) with NIR samples further shredded in a model shredder of South African design. The results were satisfactory and the factory now uses NIR analysis for cane payment (11). Similar success was reported for the NIR prediction of polarimetric sucrose and Brix in pressed juice or digested juice at Brazilian factories, where NIR analysis of cane juice became an official method for cane payment in December, 1995, in São Paulo State.

Cane quality assessment in the Louisiana sugar industry

In Louisiana, the core/press method for cane quality analysis described by Legendre (12) is an essential element of cane payment systems. In these systems, theoretical recoverable sugar or TRS is calculated empirically from the polarimetric sucrose, refractometric dissolved solids and sediment in expressed juice and the fiber content (indirect measurement) of a core sample of cane that is representative of the delivery. Similar core/press methods for cane quality analysis are utilized in many other cane growing regions (e.g., Brazil, Colombia, Trinidad and the Philippines). In general, cane payment systems based on quality, wherever they have been introduced, have been shown to effect an increase in the quality and sucrose yield in cane delivered to

the factory. However, the core/press method and any other cane quality analysis based on extraction and analysis of juice and on analysis of residual fiber are labor intensive and also subject to sampling errors.

The obvious solution to random sampling errors is to obtain more samples so as to make the samples on average more representative. In Louisiana, at least, it is not feasible to increase the sampling rate of cane deliveries to the mill; most core labs operate efficiently but at maximum capacity. Neither is increasing the capacity of the core labs for analysis a solution since it would add significantly to the mill operating costs. Hence the development and adoption of a more rapid, less labor intensive cane quality measurement would be of great benefit to both the cane grower and the mill.

Beet quality assessment by NIR spectroscopy

The feasibility studies on beet quality assessment by NIR spectroscopy are all based on the reflectance spectroscopy of beet brei obtained from the conventional tare laboratory. Although the direct analysis of single whole beets may be possible, to the best of our knowledge, this application has not been investigated. Edye and Clarke (13-16) have concentrated on beet quality assessment for grower payment and have tested several sample presentations and instrument configurations using a scanning spectrophotometer. Huijbregts and Gijssels (17) also investigated beet quality assessment by NIR using a scanning spectrophotometer with a fiber optic probe device inserted into beet brei and with a remote detector system held above the beet brei. The calibration for sucrose (based on 859 samples, range 12.5-19.5 %w/w) in the trial using the remote detector system had a standard error of prediction (SEP) of 0.31 %w/w. Calibrations for sucrose based on cuvette or cup sample presentations and scanning spectrophotometers have lower SEP's. For example, de Bruijn (18) used a sample cup presentation and obtained a SEP for sucrose of 0.13 %w/w; the correlation coefficient (R) was 0.975.

Several researchers (13-16, 18) also report NIR calibrations for the measurement of nitrogenous compounds. For example, calibration for α -amino nitrogen based on 147 samples, sample cup presentation and scanning instrument gave a SEP of 2.8 mmol/Kg (slightly higher than the reported reproducibility of the conventional method, i.e., 0.4-2.0 mmol/Kg) and a R of 0.79 (18).

Beet quality assessment in North America

The methods of assessment of beet quality for grower payment used by North American beet sugar companies are similar but not uniform. In general, beet brei prepared from tared beets (washed and in some cases topped) is analyzed for content of sucrose, cations and reactive nitrogenous compounds. While most companies measure sodium and potassium concentrations in beet brei as part of the quality assessment, at least one company uses brei conductivity as a measurement of total cations. In addition to differences in the assessed quality parameters and methods between companies, within companies biases exist between factory tare laboratories.

In the tare laboratory a production line of up to five personnel is capable of analyzing two to three samples per minute. Although the conventional analysis rate is fast the development and adoption of an equally rapid but less labor intensive NIR based beet quality measurement would be of benefit to both the beet grower and the factory. In addition, NIR technology could remove the bias between the factory tare laboratories of a single company.

Scope of this research report

This report is concerned with the development of a NIR models for direct prediction of TRS in finely chopped cane (the calculated cane quality measurement upon which Louisiana grower payment systems are based) and for prediction of sucrose and α -amino nitrogen in beet brei (beet quality measurements upon which North American grower payment systems are based). Over 3,000 spectra of finely chopped cane have been collected at a single core laboratory (one factory) in the last three cane harvests. Each year cane preparation was improved and the aims of the study were further refined to obtain results that could be presented to growers and millers to convince them of the benefits of switching to an NIR based cane quality assessment system. Progress to this end forms the first half of this report. NIR data on beet quality (reflectance spectra of brei and conventional data) have been obtained over four North American beet campaigns. In the first two campaigns, reflectance spectra of brei in cuvettes were collected at six factories. In subsequent campaigns, various instrument configurations were tested at a single factory in efforts to automate the system and decrease total analysis time. Progress to this end forms the second half of this report.

EXPERIMENTAL - METHODS AND MATERIALS

NIR analysis of finely chopped cane

Core samples from cane deliveries to a Louisiana sugarcane mill were subsampled prior to pressing and conventional analysis (polarimetric sucrose, refractometric dissolved solids and sediment of expressed juice, moisture in residual fiber). The cane subsamples were further ground (Jeffco cutter grinder, Wiley mill or Fitzpatrick mill) to a finely chopped consistency and the NIR reflectance spectra (400-2500 nm) of the finely chopped cane samples were obtained using a NIRS 6500 scanning spectrophotometer with a coarse cell and a sample transporter (Perstorp/NIRSystems Ltd., Silver Spring, Md, USA). The conventional analyses of the core samples, the calculated TRS values and the NIR spectra were used to develop chemometric models for prediction (NSAS and ISI software, Perstorp/NIRSystems Ltd.).

Determination of standard error of conventional core lab analyses

On each of three days, three core samples from the same delivery (ca. one hour before shift change) were mixed by hand and separated into four 1 Kg subsamples. The subsamples (replicates) were analyzed by the usual procedures of the core lab; the first 2 replicates by the evening shift and the last 2 by the morning shift. The results of all conventional core lab analyses were normalized to mean values and the standard error calculated as the standard deviation (σ) of the variances or residuals of replicates from mean values; standard errors calculated in this manner are directly comparable to standard errors of NIR analyses.

NIR analysis of beet brei

NIR reflectance spectra (400-2500 nm) of the beet brei were obtained using an NIRS 6500 scanning spectrophotometer and several instrument configurations (viz., a cuvette and a sample transporter, a fiber optic probe immersed into a cup of brei, and a direct light head held ca. 20 cm above a sample of brei (10-15 cm dia.)). Conventional analyses of the brei samples, polarimetric sucrose and α -amino nitrogen, and the NIR spectra were used to develop chemometric models for prediction (NSAS software, Perstorp/NIRSystems Ltd.).

RESULTS AND DISCUSSION

Cane quality assessment

During the 1993 sugarcane harvest the NIR spectra of *ca.* 200 core samples from a single factory were obtained (7). In this study the core samples from the mechanical pre-breaker (Cameco Industries, Thibodaux, Louisiana) were scanned without further preparation, and the sampling did not cover the entire harvest. The core sampler prebreaker material was too coarse, but the NSAS calibrations for the prediction of pol, Brix, fiber and TRS resulting from this preliminary study and a study at a USDA variety development laboratory (8) were sufficiently promising to warrant a more thorough investigation of this NIR application.

A more thorough investigation (with some modifications) of cane quality analysis by NIR spectroscopy was made at the same factory the following year (1994 harvest). In this study the core sampler cane was ground to a finer consistency in a Jeffco cutter grinder and *ca.* 1,000 samples were obtained at random over the entire harvest. The ISI chemometric software showed an advantage to the NSAS software for development of the statistical model. The two chemometric software packages will return the same statistical results on the training set of data. However, ISI can identify residual outliers (large differences between lab and NIR results) and spectral outliers (spectra that are significantly different from those used in the training set) but NSAS can only identify residual outliers. Spectral outliers, identified at acquisition by principal components analysis and nearness tests, can result from any incorrect packing of the sample cell (e.g., air or too much field mud at the NIR cell surface) or year to year changes in cane varieties or perhaps even changes in general weather conditions (e.g., cane from a year of severe drought followed by cane from a year of good rainfall). These spectral outliers affect the stability or robustness of the NIR calibrations and must be managed (either deleted and sample rerun or added to the training set prior to recalibration). Fundamentally, for the aforementioned reasons ISI is more suited to the reflectance spectroscopic analysis of agricultural materials where the statistical models are complex and have no physical explanation.

The statistical results of calibration or model development for the 1994 harvest are summarized in Table 1. These results and the 1995 results are reported without the removal of any residual outliers from the data set; removal of even 2 to 5% of residual outliers significantly improves the statistical results. The results from the determination of the standard error of conventional core lab analyses also are shown

in Table 1. The pressed juice pol and Brix results are comparable to those obtained earlier by S.P.R.I. (8), and those that have since been obtained by other investigators and will not be further discussed since this report is primarily concerned with the direct prediction of TRS by NIR spectroscopy. The calibration results are graphically represented in Figure 1 (for comparison with the results of the following year). The standard error for NIR prediction of TRS was comparable to laboratory standard error, and the NIR analysis was well able to differentiate cane quality over the entire range of TRS values.

Since the 1994 harvest results were very encouraging the scope of the NIR study was expanded for the 1995 harvest. Rather than collect core samples for NIR analysis at random, eight growers (of varying size of production and varying historical cane quality) were initially selected to participate in the NIR study in order to determine if a switch to NIR would affect individual grower payments. A Wiley mill was substituted for the Jeffco cutter grinder to improve cane preparation. For logistical reasons, after 14 days of sampling, the grower participant number had to be reduced to six and a more efficient Fitzpatrick mill was substituted for the Wiley mill.

By the end of the 1995 harvest over 2,000 NIR spectra had been obtained; the statistical results from a calibration model based on these spectra and the conventional determinations of TRS are shown in Table 2. The calibration results are graphically represented in Figure 2 (for comparison with the results of the previous year). A cursory comparison of statistical results from 1994 and 1995 harvests suggests that the 1995 outcome was not as good as that of 1994. In 1994 the NIR spectra were obtained by SPRI personnel and by a factory employee in a timely manner. In 1995 all spectra were obtained by a single factory employee, and on occasions when the employee was called to other duties some core samples sat at room temperature for up to two hours before NIR analysis. Significant cane deterioration and sucrose loss can occur in this time. This unfortunate circumstance would appear to explain the poorer results from the 1995 harvest.

A more thorough statistical investigation of the 1994 and 1995 data bases reveals that the results may in fact be very similar. The distributions of TRS residuals (lab TRS - NIR TRS) for 1994 and 1995 are shown in Figure 3; this is a graphical representation of the standard error of prediction values and shows a broader distribution (and greater differences between NIR and lab results) of residuals for 1995. However, the range of conventionally determined TRS values also increased from 1994 to 1995 (see Figure 4) and if the residuals are expressed as a fraction of the range of core lab TRS

values then there is little difference between the two years. This conclusion could be supported by a comparison of results from the determination of standard error of conventional core lab analyses for the two years but this analysis was not undertaken in 1995.

Certainly, the comparison of conventional TRS results for the two years leaves some doubt about the accuracy and precision of the TRS measurement (in which several rather arbitrary factors are included) and appears to place a limit on the best possible outcome of NIR statistical modeling of this data. However, the regression process for generating an NIR calibration model will, with a sufficient number of samples, automatically average out most of the random error in the lab values but incorporate any systematic lab error. The residuals in the validation step (from which the standard error of prediction is obtained) are apparent validation errors and are the sum of errors in the lab values and inherent errors (or appropriateness) of the model. Therefore, it is possible for NIR predictions to outperform conventional analyses. However, if the validation set has similar errors to the calibration set, the hypothesis that NIR predictions outperform conventional analyses can never be tested or confirmed (19). Therefore, the TRS calibration from a large training set with an apparently large standard error of prediction may be more reliably measuring cane quality than does the conventional core lab but it can not be proven by validation using only conventional core lab results.

Perhaps the most convincing argument for the superiority of the NIR method over the conventional method for cane quality or TRS measurement is that the NIR calibration for 1994 and 1995 were similar; both were PLS calibration models based on second derivative spectra (the 1994 calibration used more smoothing, i.e., fewer data points than the 1995 calibration) and the weightings and loadings were similar. This is a remarkable outcome since the cane preparation changed from 1994 to 1995, and since the lag prior to NIR analysis was variable in 1995. In fact the 1994 calibration can reasonably predict the 1995 TRS values (see Figure 5 and compare to Figures 1 and 2) even though the cane preparation changed.

Conclusions - cane quality assessment

A major contributor to the seemingly less than satisfactory agreement between NIR and conventional core lab TRS values is the error in the conventional method. The NIR method appears to be more precise than the conventional analysis, and it is certainly clear that NIR analysis is a valid assessment of cane quality.

Beet quality assessment

Figure 6 shows the results from the first two beet campaigns of NIR calibration for polarimetric sucrose in brei. The calibrations, also shown in Table 3, are based on the reflectance spectra of brei in cuvettes and represent the pooled data from 6 factories over two years (using the wet chemical data of each factory). The boxed data points in Figure 6 are from two factories that were receiving diseased beets (rhizomania); this subset appears to have a poor fit to the calibration and possibly is a major contributor to the SEP of the calibration based on the entire brei NIR spectra collection. Bias between factories also significantly contributes to the SEP.

Principal component analysis (PCA), an algorithm used to describe the variation in data sets, has been applied to the entire brei NIR spectra collection (1). PCA indicates that the spectra of brei from diseased beets are all global outliers, i.e., the spectra are significantly different from the mean spectrum of the entire brei sample collection. In fact PCA groups the entire brei spectra collection into two distinct populations, viz., diseased beets and disease free beets. The data were separated into the two groups and the results of calibration development for these groups are shown in Table 3. The consequence of these results is that we would not expect the calibration from disease free beets to measure accurately sucrose in diseased beets. However, PCA can discriminate between the spectra of disease free beet brei and diseased beet brei. Therefore, in beet growing areas where this disease is prevalent discriminate analysis using PCA data, Mahalanobis distance values (1) and calibration equations for both healthy and diseased beets could be used to develop a robust NIR beet brei analysis.

There were greater biases between factories for the measurement of nitrogenous compounds than for the measurement of sucrose. Although the data sets from individual factories were small (50 to 80 samples) the calibrations looked promising (for example, PLS calibration for α -amino nitrogen; $R = 0.795$, $SEP = 20.5$ ppm) and were similar to the results of other studies (18).

In the first two beet campaigns the cuvette system of sample presentation was used and no attempt was made to match the rate of conventional tare lab analysis (viz., 2-3 samples/min). In subsequent campaigns instrument configurations that could be adapted to on-line measurement and keep pace with the conventional tare lab were tested.

During the 1994 beet harvest 200 spectra (from 3 factories) were obtained using an NIRS 6500 scanning spectrophotometer with a fiber optic reflectance probe (FORP). Unfortunately, the high wavelength region (above ca. 2200 nm) of spectra obtained with a FORP has poor signal to noise. Since the high wavelength sucrose absorbances were not exploitable in FORP calibration development, all calibration models were based on PLS from 1500-1900 nm. The results of calibration development for polarimetric sucrose are shown in Figure 7. The PLS calibration for polarimetric sucrose, R of 0.91 and a SEP of 0.44 % w/w, was not as good as the calibration from the cuvette system (cf., $R = 0.99$, SEP = 0.40 for cuvette presentation). Repeatability (precision) studies indicated that, at least for this FORP sample set and calibration model, poor repeatability of NIR spectra substantially contributed to error. Presumably, this poor repeatability was due to beet heterogeneity and the small detector fiber bundle of the FORP. The FORP device was eliminated as a suitable instrument configuration could be adapted to on-line measurement and match the conventional tare lab analysis rate.

During the 1995 beet harvest 300 spectra (from 1 factory) were obtained using a Perstorp/NIRSystems direct light scanning spectrophotometer. This system has a light source remote to the monochromator that can be mounted above or below a sample surface. The reflected light from the sample surface is gathered and carried to the monochromator by a fiber optic bundle. This post-dispersive system has a larger sampling surface than the FORP and was developed for on-line applications. The results of calibration development for polarimetric sucrose are shown in Figure 8. The PLS calibration from 300 direct light NIR spectra has an R of 0.67 and a SEP 0.55 % w/w. It was hoped that the direct light NIR instrument might return better calibration results than the FORP (by increasing scanned sample surface area), unfortunately this was not the case.

Conclusions - beet quality assessment

The criteria for development of an automated NIR based beet quality assessment were that the calibrations should be at least as good as those from the cuvette sample presentation and the analysis should be at least as fast as the conventional tare lab. To date we have not successfully developed such an automated system. However, potential savings on labor and consumable chemicals, and the opportunity to remove measurement bias between factory laboratories still makes an NIR based beet quality assessment desirable. In future beet campaigns other instruments and sample presentations will be tested.

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Table 1. Comparison of 1994 harvest NIR calibration results with core lab error analysis results.

CONSTITUENT	PLS CALIBRATION SUMMARY				CORE LAB ERROR SUMMARY		
	Mean	R	Std. E.	MSECV	Mean	Range	Std. Dev.
Moisture	---	---	---	---	49.75	12.7	3.37
Sediment	---	---	---	---	0.013	0.047	0.0118
pol	67.43	0.94	2.00	2.10	65.9	6.5	1.75
Brix	20.46	0.95	0.58	0.70	19.7	1.9	0.63
TRS	219.31	0.84	13.31	13.62	201.22	37.14	10.91

NIR instrument could not be calibrated for moisture or sediment.

Table 2. Comparison of calibration statistics for 1994 and 1995 harvests.

CALIBRATION	1994	1995
Mean (lab)	219	204
δ (lab)	22.3	28.6
Range (lab)	144-284	99-295
Standard error (lab)	10.91	n.a.
Spectral pretreatment	2,10,10,1	2,6,6,1
Correlation coefficient	0.70	0.68
SECV	12.33	15.97
SEP	13.62	18.75 (94-20.64)

Table 3. NIR calibrations for polarimetric sucrose in beet brei.

Brei pol	Number of samples	Number of factories	Correlation coefficient	Standard error
All beets	242	6	0.96	0.6% w/v
Disease free beets	190	4	0.99	0.4% w/v
Diseased beets	52	2	0.97	0.4% w/v

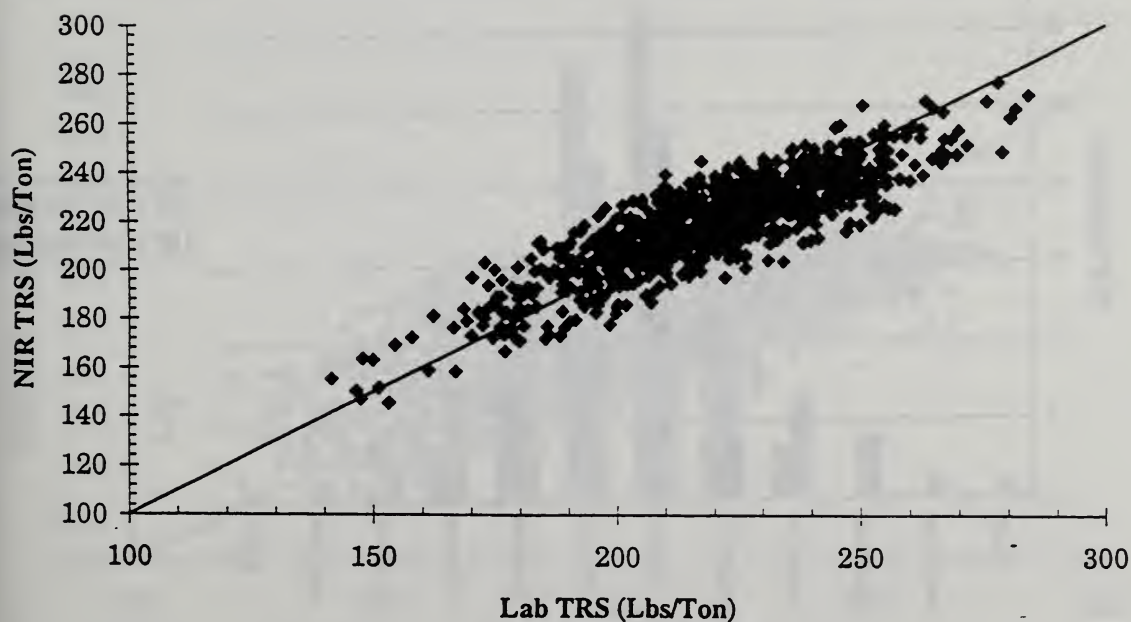


Figure 1. 1994 NIR calibration for TRS.

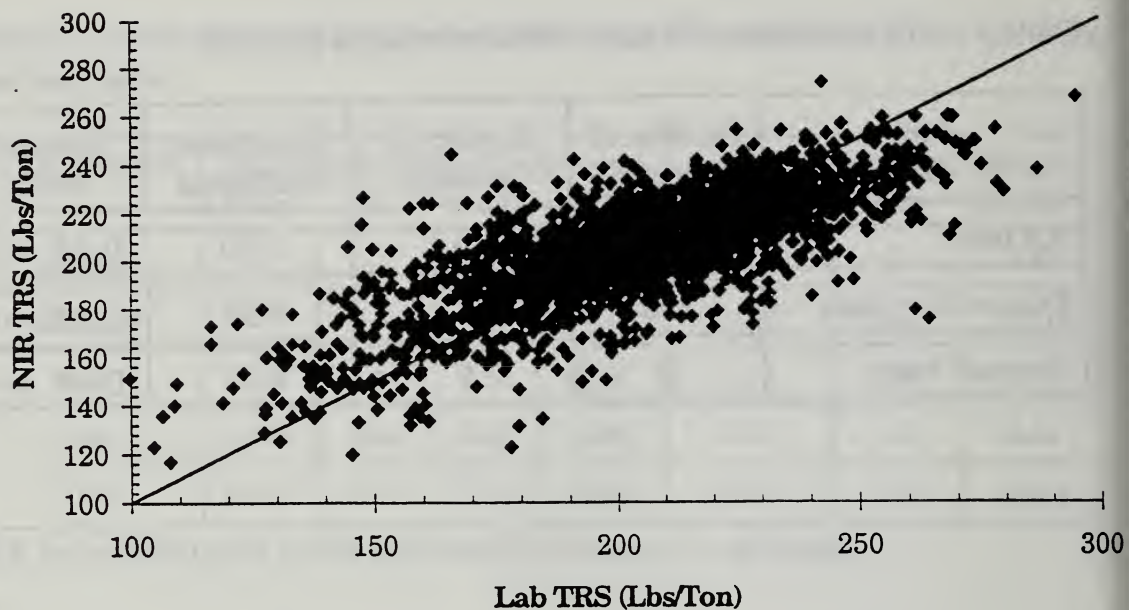


Figure 2. 1995 NIR calibration for TRS.

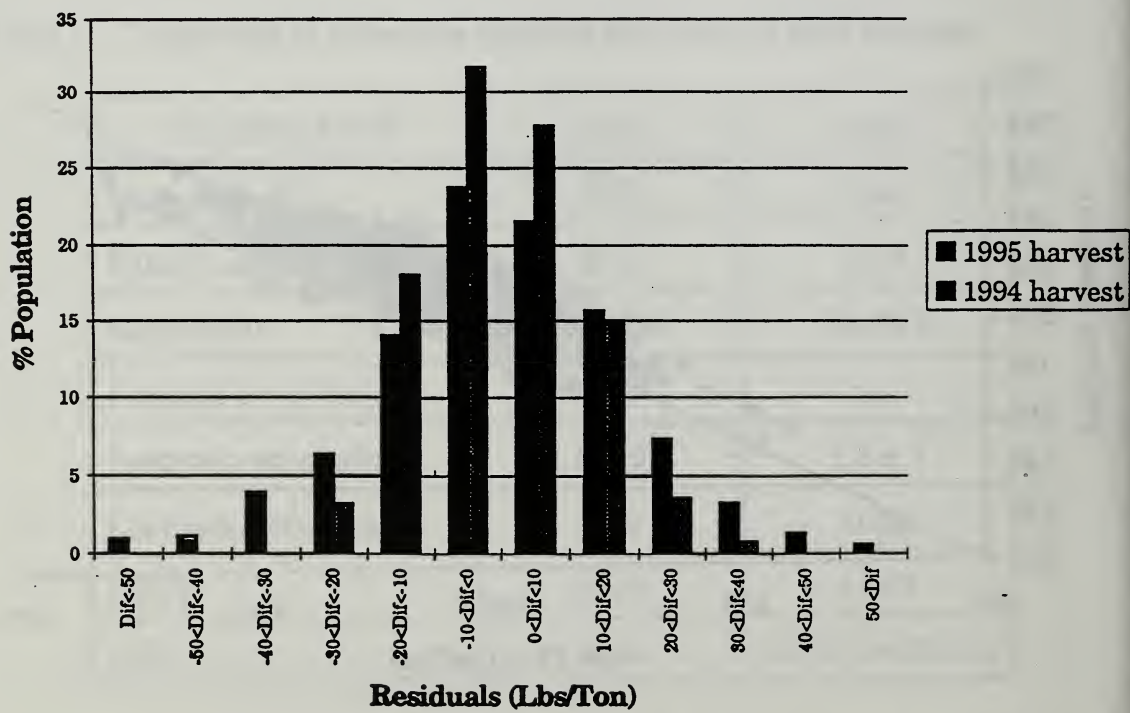


Figure 3. Distribution of TRS residuals (lab TRS - NIR TRS) for 1994 and 1995.

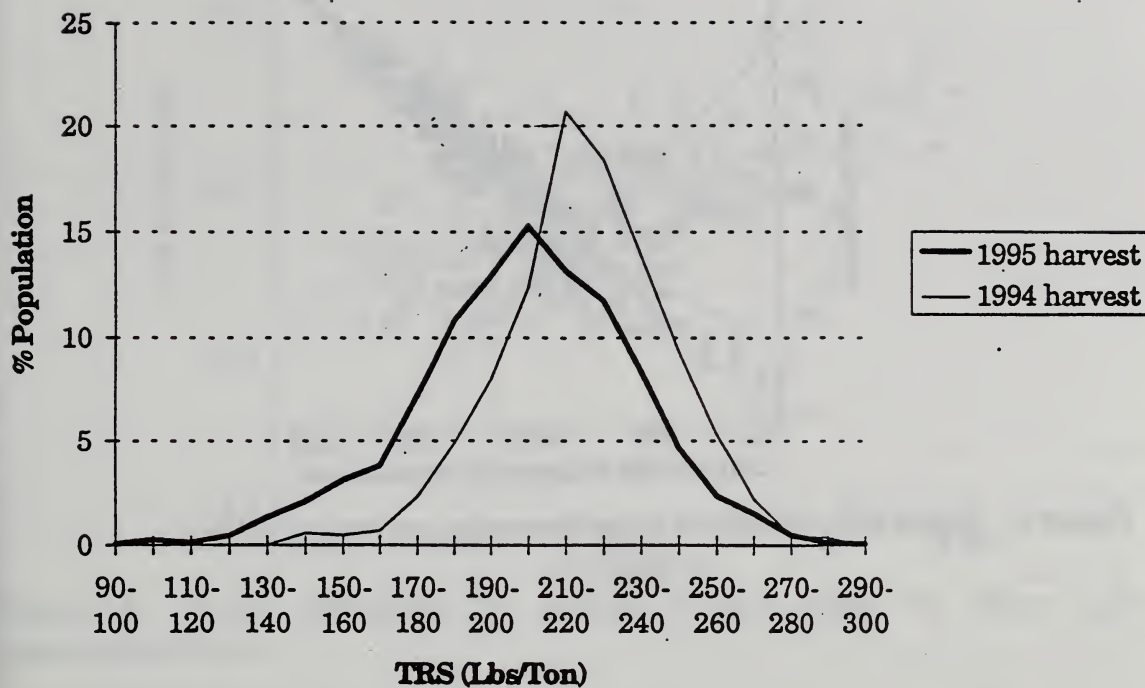


Figure 4. Range of conventional (lab) TRS values for 1994 and 1995.

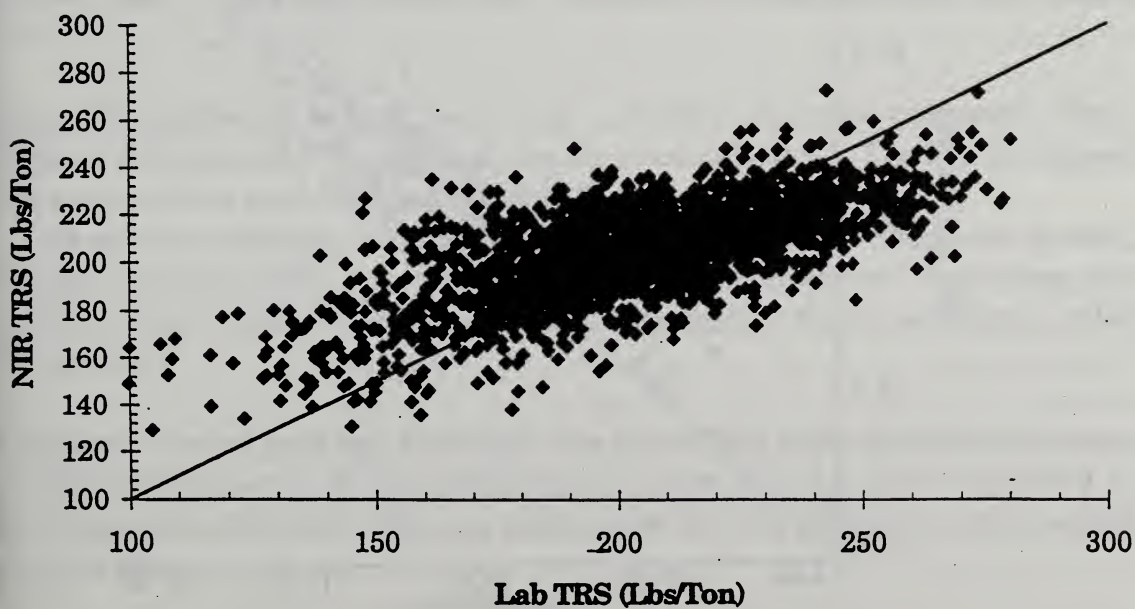


Figure 5. Prediction of 1995 TRS values based on a calibration from 1994 data.

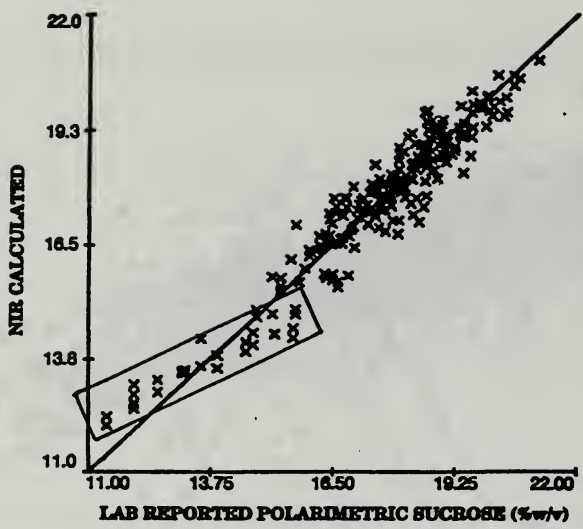


Figure 6. Global NIR calibration for polarimetric sucrose in beet brei.

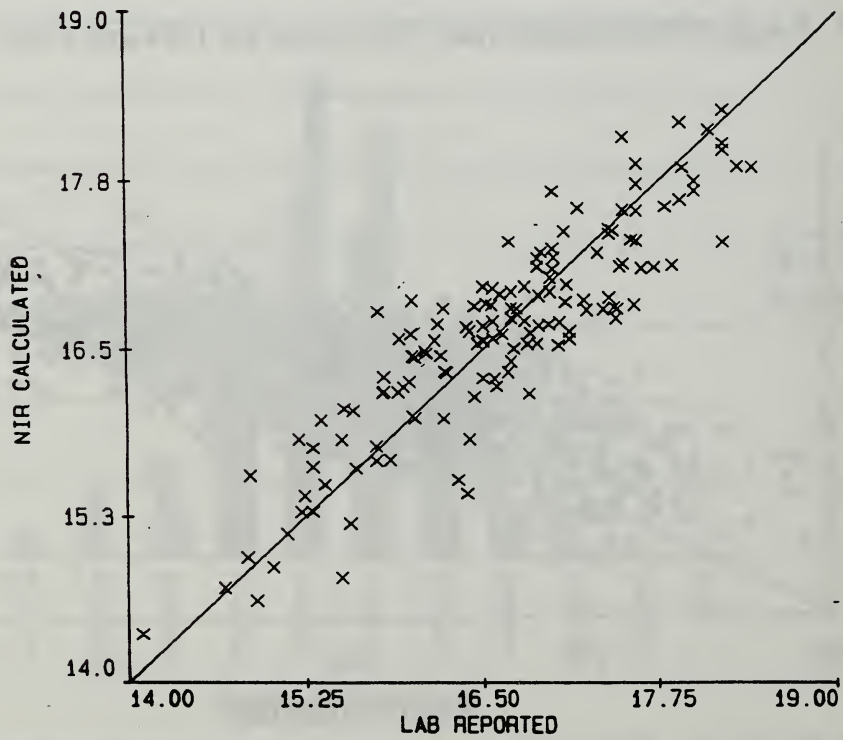


Figure 7. 1994 calibration for sucrose in beet brei using a fiber optic probe.

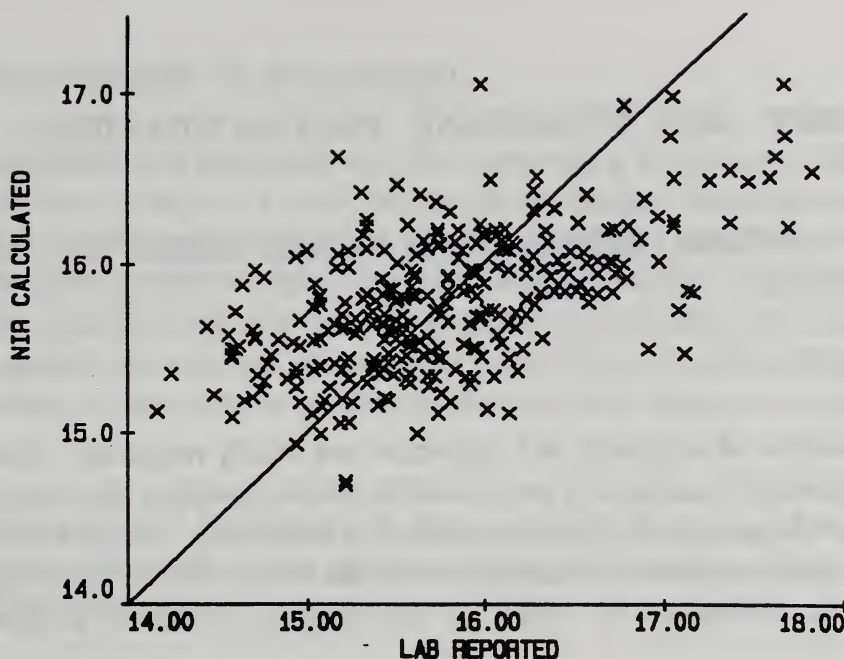


Figure 8. 1995 calibration for sucrose in brei using the direct light spectrophotometer.

DISCUSSION

Question: You think peak 2 is being converted to peaks 1A and 1B. You have isolated peak 2. Have you “cooked it up” to observe the conversion to peaks 1A and 1B?

Edye: The problem is how to “cook it up” - possibly with sugar colorants? These amounts are nanomoles - we can barely see the amounts isolated. The FACE analyses can analyze these small amounts, however.

We now have obtained milligram quantities of these peaks, to obtain nmr spectra, but the compounds gelled in the nmr tubes and would not redissolve. Since these nmr analysis require solutions, we could not run the samples. We are working to solve this problem.

Comment: Several years ago, Professor John Pazur Penn State tested the structures of ISP arabinogalactan in his immunological system, and it is not the standard 2-arabinogalactan structure. This may explain why the ratio differs from the standard stractan ratio.

Edye: Yes, this explains the difference in the ratio.

SUGARBEET AND SUGARCANE POLYSACCHARIDES: A BRIEF REVIEW

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ABSTRACT

Polysaccharides of sugarbeet and sugarcane are briefly reviewed. Both naturally-occurring plant polysaccharides and microbial polysaccharides, that are produced on the plant or in process by microorganisms, are included. Origins and causes are summarized, and properties of the polysaccharides as they affect processing and yields are outlined. Measures to inhibit or control polysaccharides in processing are discussed.

INTRODUCTION

Some plant-based (vegetative) polysaccharides of sugarcane and sugarbeet, e.g. starch in sugarcane and pectin in sugarbeet, are well documented in the literature. Their causes and origins, and controls systems to inhibit their effects on process efficiency and yield are well established (1-9). Some microbially produced polysaccharides, e.g. dextrans, are also well-documented; dextran in sugar has been the subject of a dedicated workshop (10). Other plant and microbial polysaccharides are not so well known; their origins, effects and controls are not systematically documented in the literature (12).

This summary review will briefly describe origins and causes of the well known polysaccharides from the point of view of process and yield, effects and remedies or controls. Minor polysaccharides may be mentioned, but not discussed in detail. Sugarbeet and sugarcane polysaccharides will be described in separate sections of this summary review, according to their plant origins. Microbial polysaccharides will be the subject of the third section of the review, since most of these are produced from sucrose and are common to both sugar crops.

POLYSACCHARIDES OF SUGARBEET

The polysaccharides of sugarbeet were for many years, in common with most plant polysaccharides, an art, or a craft, rather than a subject for systematic scientific investigation. Pectin was recognized as a polysaccharide (a homopolygalacturonan), but since sugarbeet pectin is highly acetylated and methoxylated and has low gelling power, was regarded only as a viscosity problem in processing and a cause of false pol. The general composition of sugarbeet polysaccharide is shown in Table 1 (4). Development of beet pulp as a dietary fiber has lent impetus to polysaccharide research.

The seminal work of J. F. Thibault (3, 4) and co-workers on sugarbeet pectins has given a better understanding to the structure of that fraction than to that of any other sugarbeet polysaccharides.

Pectin: Pectins from sugarbeet pulp range in molecular weight from 15,000-48,000 daltons, with a backbone of α -(1, 4) linked α -D-galacturonic acid residues containing a small proportion of α -(1, 2) linked of L-rhamnose residues. Side chains of neutral sugar occur in the so-called "hairy" regions of the backbone, leaving unsubstituted "smooth" areas. The side chains, usually attached to C-4 of the rhamnose residues, are of α -(1, 5)-L-arabinofuranosyl residues and β -galactosyl residues (probably 1, 4 linked). The large number of acetyl groups (up to 35% acetylation) that are responsible for the low gelling power of sugarbeet pectin, are mostly linked to C-2 or C-3 of galacturonic acid units in the "smooth" regions. Ferulic acid ester groups are attached to the "hairy" pectins, on the side chains, some esterified to arabinose units, the rest presumably to galactose units (4).

Hemicelluloses (arabinan and IBP): By definition, hemicelluloses, which are non-cellulose carbohydrate polymers, are considered to include pectins, but because pectins are such an important part of the sugarbeet hemicellulose, they are considered separately. Older literature refers to hemicellulose as made up of "arabans" and "galactans". No reports of isolation of pure compounds corresponding to polymers of arabinose or of galactose are found in the literature; these names may have been applied to extracted hemicellulose material that was shown to contain arabinose and galactose units. Extraction procedures are often difficult to reproduce; results on composition and hydrolysis products can vary widely, even when similar extraction and hydrolyses procedures are used. It appears quite probable that the hemicellulose material is galactoarabinan or arabinogalactan in nature, with varying degrees of

composition. The difficulties in separating pectins from hemicellulose compound the problems of analysis: the "hairy" regions of pectins have been shown to contain some α -(1, 5) linked arabinose chains (3, 4). Incomplete separation of various fractions is no doubt responsible for the variety of reports of composition and structure of sugarbeet hemicellulose. There is a patented isolation process for araban (more correctly called "arabinan") backbone and α -(1, 3) linked side chains (13). Because the arabinose is in the furanose form, the backbone might have a helical structure, and offer the possibility of acting as an RNA analogue for nucleoside synthesis. The molecular weight is reported variously as 8,700-24,200 daltons and 18,400-37,000 daltons (14). Again, fractionation appears to have taken place during extraction. Studies by Vogel and Schiweck (7, 15) have shown that pectins are degraded or removed almost completely during juice purification, and so the hemicellulose in thin juice is essentially pectin free. Composition, from Vogel and Schiweck (15) and from S.P.R.I. (6) is shown in Table 2; similarity of composition is remarkable in two such differently extracted isolates. Molecular weight of this fraction has been reported as between 100,000 and 300,000 (15), when material greater than 10,000 daltons is removed during the isolation process. This agrees with the molecular weight determination of pulp hemicellulose B fraction of 150,000 daltons (14). Work at S.P.R.I. on high molecular weight colorant shows a fraction at 300,000 daltons that is found in white beet sugar and process materials (6, 16); this colorant has polysaccharide nature and is thought to be related to hemicellulose material (see Figure 2).

It has been proposed (6) that this hemicellulose polysaccharide is a galactoarabinan in nature, with an arabinose backbone. Separation, isolation and purification treatments by various workers have broken these cell-wall polysaccharides, which are very soluble, into different fractions, sometimes reported as "araban" and "galactan". The galactoarabinan structure could account for the diversity in reports of polysaccharides in sugarbeet.

The observations on hemicellulose molecular weights do not account for the very high molecular weight material with colorant nature that has been observed in white sugar and molasses (6, 16). The high molecular weights of 800,000-1,000,000 daltons, are indicative of microbial polysaccharides, but these would not be expected to have colorant nature. Similar compounds have been observed in cane sugars: they may be microbial polysaccharides (dextrans perhaps) with colorant molecules esterified onto the chain.

This polysaccharide has been isolated from beet syrups and sugars, and given the trivial name "indigenous beet polysaccharide" (IBP). It may be a factor in acid beverage floc and certainly contributes to total polysaccharide and pol distortion in white sugars.

Recent studies have shown (6) that the concentration of polysaccharide in peel is an order of magnitude higher than that in the interior of the beet. In storage, polysaccharide content increased sharply in pile storage, at low humidity and temperature and less so in refrigerated storage at 4°C and high humidity.

Polysaccharide and molasses desugarization

Sugars and the materials from which they are boiled, from several molasses desugarization plants in different countries, have been studied. The resin systems used for separation of the sugar fraction appear to be effective at removing polysaccharides, and in most samples examined, level of polysaccharide in the product fraction was about an order of magnitude less (or 10% of) the level in molasses. Ion exchange resins can retain polysaccharide and apparently this adsorption is occurring in desugarization systems. Polysaccharides that are charged, such as pectin fractions, or uronic acid-containing molecules, will be more easily adsorbed than neutral polysaccharides. It may be for this reason that the second phenomenon observed occurs: the proportion of polysaccharide remaining in the product fraction that goes into the sugar crystal is higher than the normal amount. That is, the molasses desugarization resin treatment removes considerable polysaccharide from the input stream, but the type of polysaccharide remaining is more likely to go into the sugar crystal. Some examples are shown in Table 3. Investigations into the nature of this polysaccharide, and its effect which appears to be negligible on sugar quality, are continuing. The type of molasses desugarization system may affect this process considerably.

Polysaccharides and odour in sugar

There is no indication that polysaccharides of either plant or microbial origin can cause odour in sugars. However, when microbial infection (such as Leuconostoc or Bacillus species) generates polysaccharide, high odour compounds such as butyric and valeric acids or esters are often made as byproducts by the microorganisms. These "fatty acid odour" compounds are responsible for one type of odour - a sour, acid, lipid degradation product type - in sugars. A compound with this type of odour,

isovaleronitrile, has been identified as a characteristic of sugarbeet molasses alcohol (6). The presence of high levels of polysaccharides can indicate that microorganism infection is responsible for this type of odour. Examples are shown in Table 4. Wash water in white sugar centrifugals is believed to be a source of these off-odour compounds (18).

The same microbial polysaccharides occur in beet sugar as in cane sugar (see Dextran), with levans, polymers of fructose, appearing with greater frequency in beet sugar. Levans (with α -(2 \rightarrow 6) backbone linkage) can cause falsely low pol, high viscosity and turbidity; they are, however, generally removed in carbonatation.

The structure of dextran formed in sugarbeet and beet juices and syrups has been shown to be the same as that generally found in sugarcane and cane juices (19). Beet dextran can be analyzed with the same tests as cane dextran, taking caution against interference from beet glycoproteins.

POLYSACCHARIDES OF SUGARCANE

Polysaccharides that occur naturally in the sugarcane plant (Saccharum spp.) include starch and cell wall polysaccharides (arabinogalactans, arabinoxylans, indigenous sugarcane polysaccharide or ISP). Structural polysaccharides are, as usual, cellulose and hemicellulose; hemicellulose in sugarcane is composed of > 30% xylan. These structural polysaccharides are insoluble and do not generally dissolve in cane juice and so are not considered further herein. Some small xylan molecules may be solubilized in cane juice, but xylan reported from this source may be from part of a complex soluble cell wall polysaccharide.

Starch

Starch is a polymer of glucose, found in many plants, and existing in two morphological forms: amylose, a linear polymer of glucose with α -(1 \rightarrow 4) linkages, with molecular weight about 100,000 daltons, and amylopectin, a highly branched form, with a base of α -(1 \rightarrow 4) linkages between glucose molecules, and extensive branching at α -(1 \rightarrow 6) linkages with molecular weight about 40,000 Da. Both forms are only slightly soluble at room temperature, but dissolve as temperature is increased. The amylose form in solution creates high viscosity and causes increased viscosity in sugar solutions, juices, syrups and liquors. Temperatures of solubilization are shown in Table 5 (27).

Starch in sugarcane juice and cane sugars has been studied at length by many laboratories (10, 20, 21). Sugarcane starch is located in the nodes of the cane stalk, and not in the internodal areas, so any growth factor that increases the number of nodes per stalk will increase the level of starch in juice. Starch is present at higher concentrations in leaves and in growing tips than in the stalk, so increase in inclusion of leaves and tops with cane delivered to the mill will increase starch concentration in juice. Starch exists as granules, of amylose and amylopectin, in the plant. Granules are extracted into the juice, and dissolved as juice is heated. Elevated temperatures of maceration water will cause increased extraction of solubilized starch, relative to sucrose, in the last mill. Table 6 outlines the extraction and solubilization patterns of starch (21).

Normal granules of sugarcane starch are 3-8 μ in diameter, with about 15-20% amylopectin content. A second type of starch granule has been observed, first in raw sugar and then in juice. A smaller granule, size averaging 2 μ diameter, which is not solubilized and progresses through processing in granular form.

The role of starch in the sugarcane is to be a form of storage of energy, alternate to sucrose. Starch is synthesized by the plant in the daylight. The amounts of starch in cane are strongly dependent on cane variety, and on stage of maturity of cane. Amounts or concentrations of starch in cane vary up to 2500 ppm on solids in juice (21); no strong correlation with other varietal factors has been observed. Concentrations are higher in immature cane, decreasing as cane reaches maturity.

Effects of starch in processing

The immediately noticeable effect of starch is increased viscosity. Viscosity increase in syrup slows down crystal growth, increases pan times, lowers yield and can cause purging difficulties in the centrifugals. Viscosity increase in refinery liquors causes slowdown of filtration, in addition, decreasing throughput. The effect is more serious in carbonatation process refineries, where filtration is a major process step. Starch, however, can be removed in part in carbonatation, where it can be trapped in the growing calcium carbonate crystal. Phosphatation clarification is even more effective at reducing starch concentrations. The presence of small undissolved starch granules increases filtration problems by blinding filter cloths and screens. The effect of increasing viscosity with increasing temperature often causes confusion in process. The processor's normal response to a high viscosity syrup is to increase temperature,

but when starch is causing high viscosity, an increase in temperature will further increase viscosity.

Starch has a positive polarization, about three times that of sucrose, or about 300°S , and so can cause pol distortion in juice or sugar if the starch is solubilized. Starch can also contribute to alcoholic beverage floc, a haze or turbidity that can form in sweetened alcoholic beverages, e.g. creme de menthe, orange liqueur. Undissolved granules contribute to turbidity and sediment in sugars.

Starch removal

Starch can be removed to some degree (50% to 60%) in carbonation plus filtration, or 60% to 70% by phosphatation. The most effective treatment, however, is by amylase enzyme, starch degrading enzyme which breaks up the starch molecule into smaller chains of glucose (but only a small amount of molecular glucose) (20, 29). This treatment reduces the viscosity problem, because the small chains do not have the viscosity increasing effect of amylose. The enzyme products, starch fractions or oligosaccharides each have a chemically reducing end, which causes high results in reducing sugars analysis.

Other polysaccharides of sugarcane cell wall polysaccharide - ISP

Sugarcane soluble cell wall material appears to be present in all sugars, raw, white and refined. Major components of this cell wall hemicellulose are galactose and arabinose. Roberts has isolated this material from fresh sugarcane and from many sugars and process materials; Roberts has identified the polysaccharide as an arabinogalactan with galactose backbone, probably β -(1-3) linked, with arabinose branches at the 6-position (1, 6). There is about 8% glucuronic acid present, probably as a substituent on the 6-position of galactose. The polysaccharide was given the trivial name "Indigenous Sugarcane Polysaccharide" or ISP. It has a negative specific rotation (about -50°), and so can lower pol readings. Molecular weight ranges from 100,000 to 300,000 Daltons. The ISP is very soluble and is the predominant polysaccharide in normal fresh cane juice (except for high starch varieties), in concentrations ranging up to 6,000 ppm on solids. Composition has been found variable, probably because of variation in extraction procedures. ISP is linked to phenolic and flavonoid residues in the cane plant, and is thought to be one source of these colorant and precolorant molecules and also a means by which these residues are carried through processing (5).

ISP has been found to be one of the factors causing acid beverage floc in soft drinks (5, 17). ISP is observed to develop a negative charge at pH below 4; any protein will develop a positive charge at pH below the isoelectric point (usually 3.5-4.0), and can, through charge attraction, forms a complex with ISP. This can initiate floc formation. Suspended solids, colloidal species and high molecular weight dissolved material can become trapped in the floc network and enhance the appearance of the floc, as outlined in Figure 3. ISP is extremely soluble, and travels through processing to raw sugars (up to 3,000 ppm) and white sugars (up to 1,500 ppm). It can be removed by tight filtration at temperatures below 65°C (22). Such a filtration process appears to be a way to inhibit acid beverage floc formation. ISP will also, as will all alcohol insoluble polysaccharides, contribute to alcohol beverage floc.

Phytoglycogen

Phytoglycogen is a small (40,000 Daltons) polymer of glucose with α -(1-4) linked backbone and ~ 12% α -(1-6) branches. It is apparently analogous to glycogen in animals, and has been found in sweet corn and sweet sorghum, close botanical relations of sugarcane. It is extremely water soluble, and has a specific rotation of +120°. Other than its potential for distortion of pol by elevation, it does not appear to have any negative effects on processing. It is present at up to 2,000 ppm on solids in cane juice (5, 23).

Galactomannans and others

Other polysaccharides, notably galactomannans reported by Miki and colleagues at the Japan Sugar Refiners' Association (5), have been reported from sugar and sugarcane from specific areas.

MICROBIAL POLYSACCHARIDES

Dextran

Dextran is a microbial polysaccharide, formed by the microorganism Leuconostoc mesenteroides (and other Leuconostoc species, but this is the common bacteria in sugar crops). The Leuconostoc eats sucrose, forms a polymer from the glucose residues in sucrose as an exocellular polysaccharide and leaves remaining fructose in solution. The presence of dextran indicates specific loss of sucrose. The dextran polymer found in cane and beet is of high molecular weight, 2×10^6 Da or more in its

native state, and has an $\alpha\text{--}(1\text{--}6)$ linked glucose backbone, with about 5% branching as $\alpha\text{--}(1\text{--}3)$ or $\alpha\text{--}(1\text{--}4)$ linkages. It is very soluble in water (or sugar solutions) and increases viscosity rapidly with increasing concentration. Leuconostoc forms dextran most rapidly in dilute solutions of sucrose (up to 25% sucrose) at pH between 5 and 7 and at rates increasing with temperature between 20°C and 50°C. The infection takes place, generally, on the sugarcane, where the bacteria enter cane at any open wound. Wounds caused by pests or disease damage, animal mutilation, harvesting, burning, mechanical damage (tractors or trucks running over cane) all provide exposed tissue for entry of the Leuconostoc organism. Population of the organism, which is a facultative anaerobe, increases most rapidly in anaerobic, warm, moist conditions, such as when cane is covered with mud and stored in piles in warm, wet weather.

There are two factors to be considered in dextran production: one is the number of organisms present, and the second is the rate at which each organism consumes sucrose and produces dextran. The population of organism on cane entering the factory determines the level of population in juice, where dextran can be produced extremely rapidly. Studies have shown that cane deteriorated from remaining in the field after burning or harvest (or after freeze damage) or other mechanical damage will have highest organism population if cane is wet and muddy. If wet, mud covered cane is then put into piles, when the center of the pile can become warm, populations increase rapidly. On the other hand, dry relatively clean cane, in dry, possibly breezy areas, e.g. Natal, where cane is hand cut and placed in small piles of only a few tons, is likely to have a very low population of dextran-forming organisms, and so produce little dextran in juice or in factory.

The Leuconostoc population, and dextran production, will vary directly with the area of exposed tissue: therefore, whole stalk cane; without other mechanical, weather or pest damage, will have a smaller exposed area than billeted cane, and long billets a smaller total area than short. On a 6 ft cane stalk, 9" billets will have more than 50% greater exposed area than 15" billets. This is a factor for serious consideration in areas where mechanical chopper harvesting is becoming more widespread, and dextran levels in juice are a problem.

Major factors affecting dextran levels in cane entering a factory are:

1. Time between harvest and delivery, or between harvest-to-burn, and burn-to-delivery where cane is still burned. With increasing time, dextran levels increase asymptotically (9).

2. Ambient temperature at harvest and delivery times. With increasing temperature, dextran levels increase dramatically (9). Nighttime temperatures during the period when cane is stored in carts or caneyard, are important.

The best conditions for low levels of Leuconostoc organism and of dextran in cane arriving at the factory are: cool, dry weather; clean, undamaged cane with as few wounds as possible; and short delivery times.

Dextran can also be formed at the factory, first in the cane yard, where minimum storage times and amounts should be enforced.

Dextran can form in juices and waters containing sucrose across the mill, until juices are heated. Dextran can form in syrup holding tanks, in valves and pumps, anywhere dilute solutions (<30%) of sucrose are at temperatures below 50°-60°C. Water condensing on top of syrup or molasses storage tanks can give a source for dextran production, as can sweet waters, or syrups held over for recycling.

Dextran control and removal

The best control for dextran is the inhibition of its formation. Good coordination of harvest and delivery with minimum holding times after burn or harvest, are important for all cane but especially for chopper-harvested, or billeted, cane, with its greater amount of exposed tissue. But sometimes, weather (rain, freeze) prevents efficient delivery, and dextran levels are high in cane arriving at the mill. Then the use of dextranase enzyme is an effective control (9). The effect of dextranase enzyme is shown in Figure 3. Dextranase enzyme, used either on juice or in the evaporators, will break large dextran polymers down to smaller molecules. Viscosities will drop, massecuites recover movability and crystal distortion decrease when the large dextran molecules are broken down. The enzyme products are generally too small to be read by a haze test, and so do not analyze as dextran.

To control formation of dextran in the mill and boiling house, there are available biocides which kill the Leuconostoc organism and prevent further production of dextran in cane juice. The use of low pressure streams for frequent cleaning of the

mills and nearby areas is also recommended. Attention to good hygiene throughout the factory will keep dextran levels low under regular conditions. The introduction of deteriorated, even rotted, cane calls for extraordinary measures with increased biocide treatment and enzyme application.

Other microbial polysaccharides

Other organisms as well as Leuconostoc can produce microbial polysaccharides. Levans, which are polymers of fructose, analogous to the dextran polymer of glucose, are formed by Bacillus spp., but have been reported more frequently in sugarbeet than in sugarcane (1, 5, 24, 25). Levans, β -(1-2) polymers of fructose, are water soluble and so travel through process; they increase viscosity and add to alcoholic beverage floc. There are no reports of control by levan degrading enzymes in process, though such enzymes have been used for structural determination of levans. Preparation of a levan from sucrose by B. polymyxa, for use as an industrial polysaccharide, is reported (24, 25).

Yeasts are present in the mixture of bacteria, molds and fungi on cane, as on most crops. Reports that ethanol is an indicator of cane deterioration, or staleness (rather than dextran), in South Africa indicate that yeasts may be the prevailing microorganism in that area, rather than Leuconostoc. Dextran can still, however, be formed in mills and factories. Ethanol is formed by yeasts, notably Saccharomyces cerevisiae, which also form polysaccharides, polymers of mannose, called mannans. The presence, activity and effects on processing of these yeast mannans are under investigation at S.P.R.I.

Effects on white sugar quality

All high molecular weight polysaccharides can cause floc in alcoholic beverages (cordials, liqueurs, cough syrups) because they (including dextrans, levans, cell-wall polysaccharides) are precipitated by alcohol and form haze or turbidity in alcohol mixtures. Polysaccharides in crystal sugar tend to retain moisture, and so lower storage quality of sugar, by making the drying process more difficult. Normal drying and conditioning times for white sugars will not be long enough for sugars with unusually high levels of polysaccharides, and unexpected caking and hardening can occur in subsequent storage. Product color may increase, in crystallization and/or in storage, from polysaccharide related causes: color associated with dextran molecules may be chemically released to cause yellowing, and color can form readily from

fructose and acid byproducts of dextran formation. Dextran itself, when present in high concentrations, gives a dull, greyish appearance to sugar crystals, taking away the sparkling whiteness.

Another product quality problem, from dextran especially, is crystal distortion: crystal elongation occurs during crystallization, giving so-called "needle grain" crystals, which can block centrifugal screens and impede purging. When a sugar with high levels of dextran is used to make hard candy (sweets), distorted crystals will form in the solid candy, distorting the shape of the candy and causing packaging difficulties - the distorted candy does not fit the package.

Polysaccharides can add to acid beverage floc in soft drinks. Although, in cane sugar, only I.S.P., the cell wall polysaccharide, can initiate floc (along with dissolved protein), dextran or starch molecules present can enhance the appearance or heaviness of the floc.

SUMMARY

This paper has presented a general description of the major polysaccharides of sugarbeet and sugarcane, both plant and microbial. Their effects on sugar manufacture, on processing and products, and measures for their control or elimination, have been discussed.

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Table 1. Sugarbeet polysaccharides: general composition.

Polysaccharide	% content, beet pulp
Cellulose	20-25
Hemicellulose	30-40
Pectin	25-30
Starches, gums, mucilages	<3

Table 2. Composition of sugarbeet hemicellulose (without pectin), showing normalized percentages.

Sugar, %	Thin juice (4)	Raw juice (9) Depectinated
Arabinose	48.8	44.8
Galactose	36.2	34.1
Rhamnose	3.3	N.r
Mannose	1.3	0.93
Xylose	0.3	2.4
Glucose	8.8	17.69
Uronic acid	1.4	17.7

Table 3. Polysaccharides in molasses desugarization, ppm on solids.

Desugarization	Factory 1	Factory 2	Factory 3
input	not available	5707	1055
output	766	344	230
sugar	150	312	151
Standard			
thick juice	1218	1850	1930
sugar	53	121	153

Table 4. White sugars with odor problems and some of their properties.

Sugar	pH	Odor	Color (ICU)	Polysaccharides (ppm)	Phenolics (ppm)
Problem IV	6.11	Strong beet odor	22	334	49
Control IV	5.88	-	37	82	46
Problem V	6.86	Strong odor/also sour	28	163	28
Problem VI	6.04	Fatty acid, cheesy	17	312	<1
Control for V and VI	6.86	-	37	131	26

Table 5. Temperature range for loss of birefringence (or solubilization) of cane starch in sucrose solution of ranging Brix (27).

Temperature, °C, for % loss of birefringence			
Brix	2%	50%	98%
0	56	58	59
10	58	61	65
20	60	64	70
30	65	70	74
40	73	76	79
50	81	85	88
60	93	95	97
70	103	106	--

Table 6. Extraction of soluble starch. All starch in mg/kg on solids.

Factory A	Total	Soluble	% Soluble
Crusher juice	1994	232	11.6
Residual juice	1158	1032	89.1
Dilute (mixed) juice	1643	661	40.2

Factory B	Total	Soluble	% Soluble
Crusher juice	609	494	81.1
Last mill juice	905	905	100.0
Dilute (mixed) juice	600	486	81.0

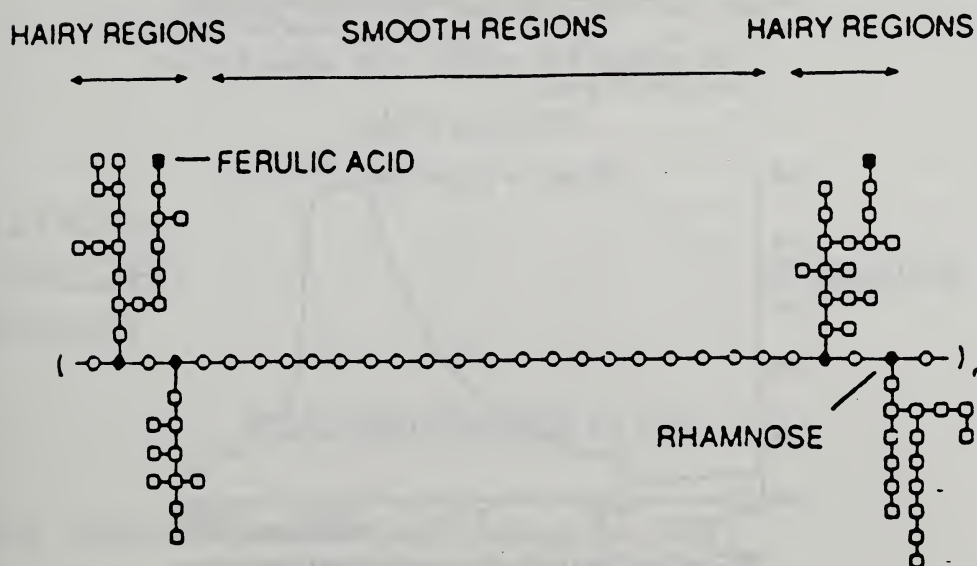
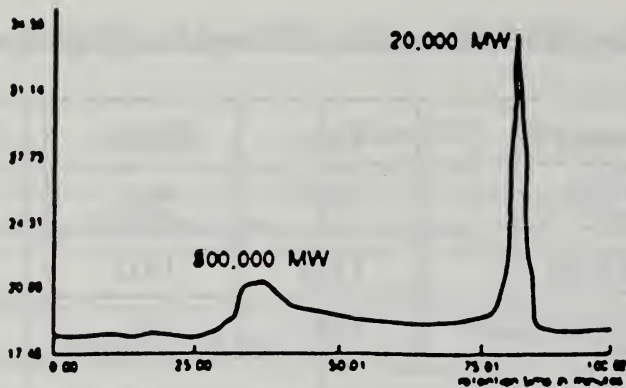
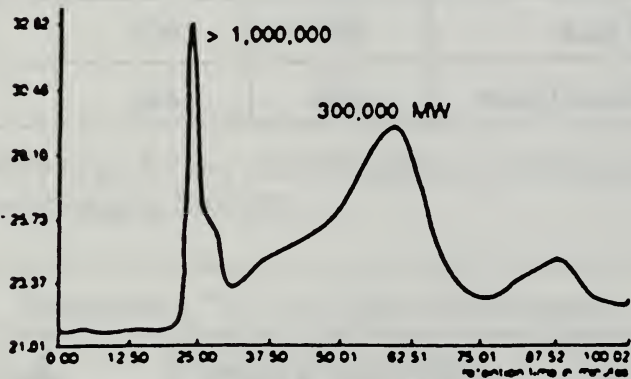


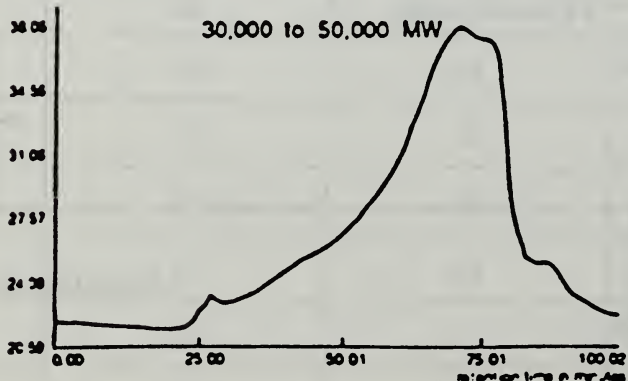
Figure 1. Schematic structure of sugarbeet pectin (from Thibault) (4).



GPC profile of high molecular weight colorant from beet white sugar (25 g sugar)



GPC profile of high molecular weight colorant from beet raw sugar (20 g sugar)



GPC profile of high molecular weight colorant from molasses (1g molasses)

Figure 2. GPCs of white beet sugar, raw beet sugar and molasses (6).

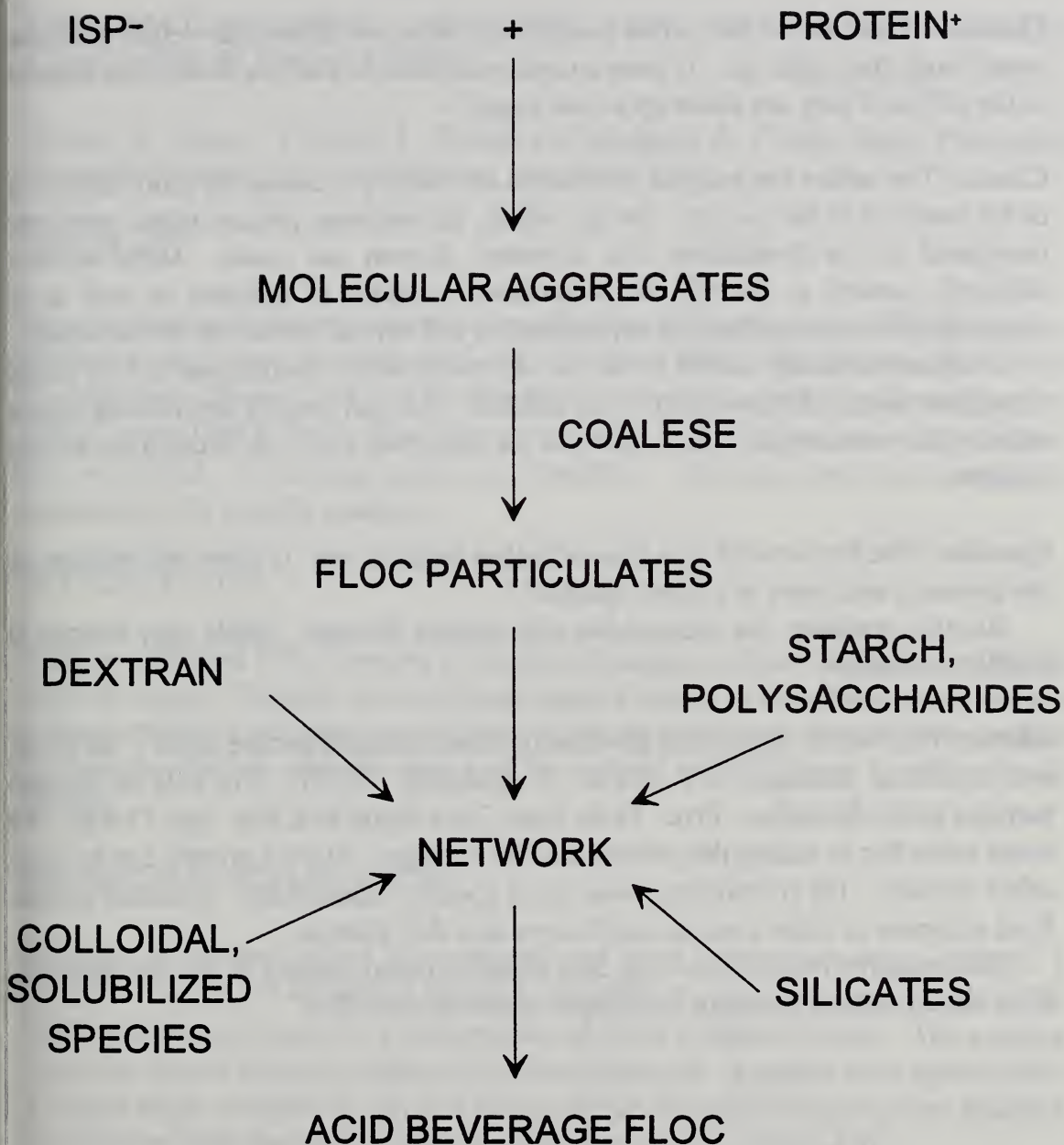


Figure 3. Outline of formation of acid beverage floc (ABF).

DISCUSSION

Question: There are no tests so far available for these low molecular weight dextrans, even though they affect pol. Is there an optimum place to add the dextranase enzyme in the mill so it may not show up in raw sugar?

Clarke: The earlier the enzyme is added in the factory process, the more likely it is to be removed in the factory. On the whole, the enzymes present minor problems compared to the devastation that untreated dextran can cause. More work is definitely needed to identify the breakdown products of amylase as well as of dextranase, and their effects on crystallization and crystal formation and structure.

Amylase is usually added in the second evaporator. Dextranase can be added anywhere: to mixed juice or on to the crusher. The more stable dextranases can be added to the vacuum pan - the A-pan and the effect will carry on through the boiling system.

Question: The floc network you showed makes sense to me. Is there information on the presence and types of protein species?

Another question: the melanoidins also contain nitrogen. Might they interact in the floc network?

Clarke: Yes, there is data on the proteins that can be found in cane sugar - we found a ribonuclease (Roberts, E. J. and M. A. Godshall. (1976). The role of charged particles in floc formation. Proc. Tech. Sess. Cane Sugar Ref. Res. pp. 73-81). We could make floc by adding this protein and ISP to sugar. But the protein can be from other sources - the ribonuclease was not a specific requirement. Residual protein from enzymes or other sources could serve as a floc initiator.

With regard to melanoidins - yes, they probably could interact in the floc network. That would explain the color sometimes observed with floc.

POSTER

**CHROMATOGRAPHIC ANALYSIS OF SUGARCANE JUICE:
COMPARISON OF METHODS**

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ABSTRACT

Sugarcane juices and syrups were analyzed by five different cationic HPLC systems. Columns used were: BioRad HPX-87C, Bio Rad HPX-87P, Waters μ Bondapak Carbohydrate, Waters C-18, and Shodex 801-S. Samples were also analyzed by anion HPLC, using HPAE-PAD. Results are compared, and differences among analyses are explained. Co-eluting peaks are identified. Systems are recommended as appropriate for specific analyses.

INTRODUCTION

Problem: Different HPLC systems of analysis of sugarcane juice each give a range of different values. Samples were analyzed under a variety of conditions using various columns that have industrial applications. Each set of columns gives different values of sucrose, varying with the nature of the column and the eluant specific to each system.

MATERIALS AND METHODS

Samples of clarified mixed juice, second and third evaporators were made to a concentration of 2% of Milli-Q-Water for the HPLC system. Samples used for the HPIC system were made to a concentration of 0.2% in Milli-Q-Water. The samples were then filtered through a Millex 0.45 micron filter unit. Samples were injected into a Waters Sugar Analyzer by way of a Waters WISP Automatic Injector, then detected on a Waters High Resolution Differential Refractometer, Model 410.

SPRI

Column Name:	Type:	Eluant:
HPLC		
BioRad HPX-87C	Calcium cation	40 mgCaOAc/L Milli-Q-Water
BioRad HPX-87P	Lead cation	40 mgCaZOAc/L Milli-Q-Water
Waters μ Bondapak	Silica based/ amino bonded	80% Acetonitrile/Milli-Q-Water
Waters C-18	Silica based/ reverse phase	40 mgCaOAc/L Milli-Q-Water
Shodex 801-S	Sodium cation	40 mgCaOAc/L Milli-Q-Water
HPIC		
Carbo-Pac PA1	Hydroxide anion	100mM NaOH/Milli-Q-Water

RESULTS

Sample #	HPX-87C	HPX-87P	μ Bondapak	C-18	801-S	Avg.	Pol 880nm	Pol 589nm
1	19.87	19.72	19.61	20.05	19.12	19.67	19.12	18.88
2	34.00	33.43	33.88	32.20	32.32	33.17	32.24	31.52
3	28.80	29.45	30.01	27.96	28.30	28.90	29.08	29.20
4	50.44	53.92	54.87	50.40	51.97	52.32	46.20	52.16
5	7.70	8.00	8.02	7.63	---	7.84	7.92	8.80
6	---	57.79	57.37	55.43	56.62	56.80	55.12	54.64
7	33.13	33.80	---	---	34.68	33.87	31.44	32.48
8	46.36	47.54	50.52	49.90	49.78	48.82	52.60	52.56
9	55.02	54.08	---	56.84	58.22	56.04	53.96	53.60
10	10.68	10.53	10.82	9.83	11.05	10.58	10.44	10.48

All results in % w/w.

Cross correlations

	HPX-87C	HPX-87P	μ Bondapak	C-18	801-S	Pol 880	Pol 589
HPX-87C	1						
HPX-87P	0.99	1					
μ Bondapak	0.99	0.99	1				
C-18	0.99	0.99	0.99	1			
801-S	0.99	0.99	0.99	0.99	1		
Pol 880	0.98	0.98	0.98	0.98	0.98	1	
Pol 589	0.98	0.99	0.99	0.99	0.99	0.99	1

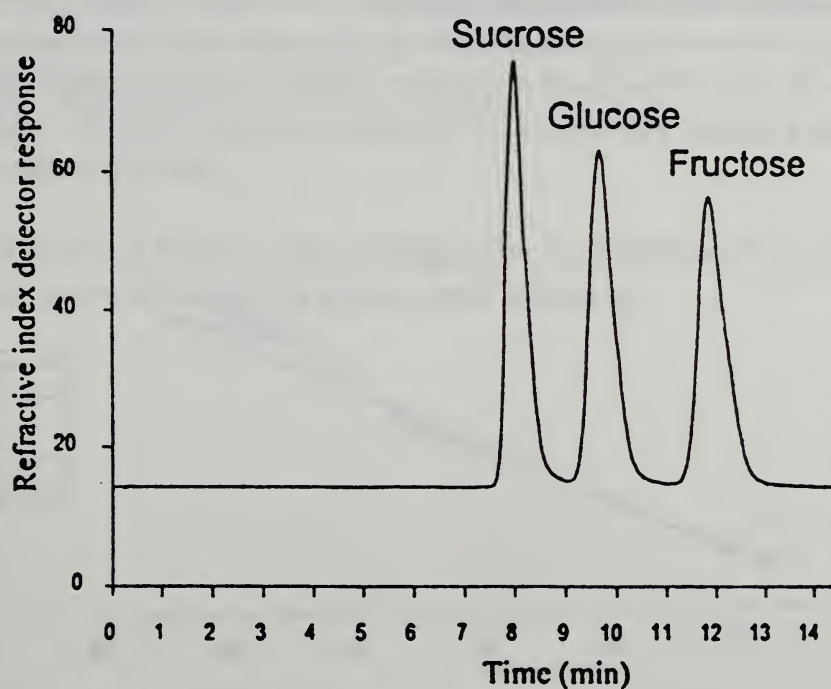


Figure 1. HPX-87C standard curve.

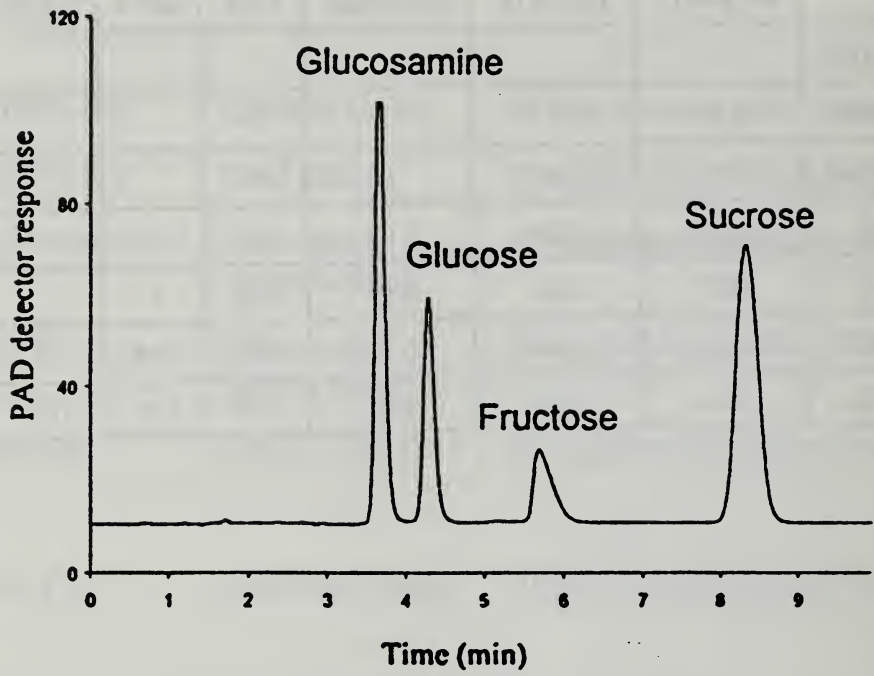


Figure 2. Carbo-Pac PA1 standard curve.

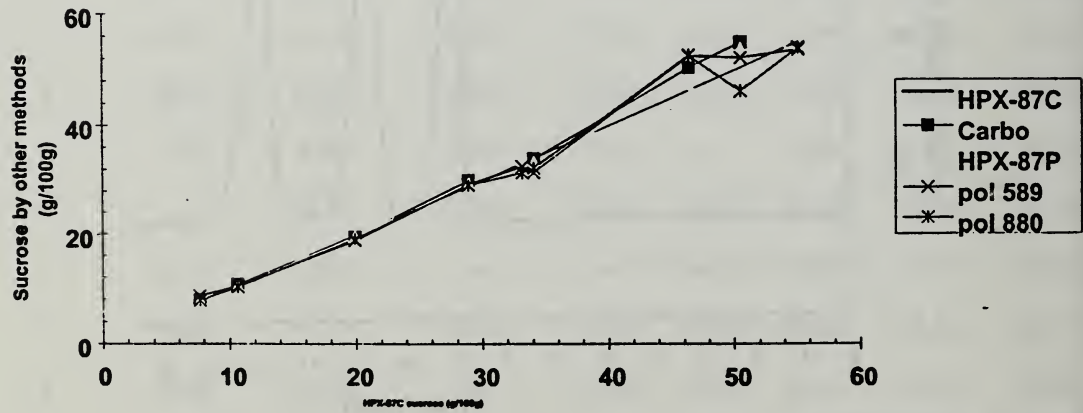


Figure 3. Comparison of sucrose analyses to HPX-87C results.

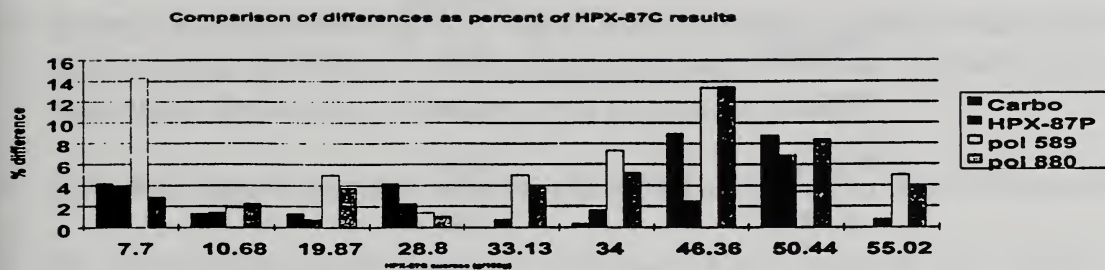


Figure 4. Comparison of differences as percent of HPX-87C results.

ERROR IN MEASUREMENT

Dionex (HPAEC-PED) values are considered the most accurate in chromatography. To calculate error: 10 vials were made up with the concentrations of 0.02g of sucrose and 0.002g of glucosamine in 100mL volumetric flask and brought to volume with Milli-Q-Water. They were then run on the HPIC to estimate accuracy and precision. The results are in g/100mL.

Because HPAEC is at least one order of magnitude more sensitive than cation HPLC, instrumental errors are magnified greatly upon scaling up.

Carbo-Pac correlations

	Carbo-Pak
HPX-87C	0.95
HPX-87P	0.94
μ Bondapak	0.94
C-18	0.96
801-S	0.95
Pol 880nm	0.97
Pol 589nm	0.96
Carbo-Pak	1.0

Sample	Dionex integrator	HPIC integrator
1	0.0232	0.0232
2	0.0227	0.0226
3	0.0217	0.0217
4	0.0220	0.0219
5	0.0221	0.0222
6	0.0221	0.0225
7	0.0232	0.0234
8	0.0236	0.0259
9	0.0252	0.0251
10	0.0230	0.0231
Average	0.0229	0.0232
S.D.	0.0010	0.0013
% error	4.27%	5.58%

5% is standard error for most chromatographic methods.

CONCLUSIONS

- Errors in all systems are higher at higher concentrations, probably because of (1) dilution error (2) error in scaling up results and (3) higher concentrations of substances that co-elute (interfere) with the sucrose peak.
- HPX-87C system maintains integrity (no interferences) up to 30% w/w sucrose.
- No one chromatographic system is consistently higher or lower than another.
- All systems correlate well with one another, except that the high wavelength pol (880 nm) shows a trend to slightly lower correlation with all HPLC methods.
- When changing HPLC analysis systems for cane juice or syrups the new system must be calibrated independently and correlated against the previous system.

POSTER

COMBINATION OF FLOCCULATION AND CROSSFLOW FILTRATION FOR REMOVING COLORED IMPURITIES IN RAW CANE SUGAR SOLUTION

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ABSTRACT

In the cane sugar industry, membrane filtration has been increasingly investigated as a purification process. It is well established that colored compounds hinder raw cane sugar refining. In this paper, the combination of flocculation and micro/ultrafiltration processes was studied on raw cane sugar solutions in order to improve permeate quality in terms of turbidity and coloration. The pilot tests conducted on raw sugar solutions at 50 Brix indicated that the coupling of these two processes led successfully to a permeate decolorization of 50% at a 60 l/h.m² permeate flux. This result is considerably higher than the usual decolorization rate given by micro/ultrafiltration alone.

INTRODUCTION

The cane sugar refining process is mainly focused on removing colored compounds which alter the quality of the final product. Traditionally, the refining process includes affination that removes 40 to 50% of the colored impurities, and carbonation or phosphatation that eliminates 40 to 60% of the remaining color. Microfiltration and ultrafiltration techniques have been investigated as an alternative to both affination and purification stages ^(1, 2, 3). Moreover, ultrafiltration performed on cane juice, which has been developed considerably over the last few years by Applexion, has allowed the production of a very high quality sugar ⁽⁴⁾.

The objective of the present work was to optimize cane sugar permeate quality while attaining economically viable permeate fluxes. To achieve this goal, flocculation was coupled with micro/ultrafiltration to remove efficiently color compounds ⁽⁵⁾. Several types of membranes covering a wide range of pore size were investigated to assess

the potential of the combination of flocculation and micro/ultrafiltration processes to purify raw cane sugar solution.

SCOPE OF THE PROJECT

Microfiltration studies on raw cane sugar solution showed that microfiltration removed efficiently turbidity whereas color was eliminated to a lesser extent ⁽⁶⁾. This low color removal can be overcome by properly pretreating the raw sugar solution before micro/ultrafiltration. In a sugar refinery, phosphatation treatment is frequently used to clarify washed sugar liquor. The phosphatation process consists of producing calcium phosphate precipitate that entraps suspended and colloidal material such as bagasse particles, proteins and polysaccharides. In addition, 20-35% of the color can be removed, depending on the amount of phosphoric acid used ⁽⁷⁾. To improve decolorization without using an excessive amount of lime that would raise scale problems, phosphatation can be supported by a flocculating agent. Since most of colorants contain negative charges, a cationic polymer could attract colorants and generate large agglomerates. Incorporated in large particles, colorants should be more retained by the micro/ultrafiltration membranes. At the same time, increasing the size of particles could limit membrane fouling and therefore, could promote higher fluxes. A cationic quaternary ammonium was therefore selected as an extra decolorizing agent. Overall, the present flocculation operation consisted of mixing three compounds which are: a quaternary ammonium, lime milk and phosphoric acid. In this way, a combination of flocculation and micro/ultrafiltration on raw sugar syrup would result in producing a sparkling syrup with reduced color and increased purity.

EXPERIMENTAL PROCEDURES

Flocculation. A 30-liter batch of 50 Brix solution was prepared from raw cane sugar and heated to 80°C. To the batch was added the proper doses of the flocculating agent which was previously determined by conducting jar-tests. The mixture was gently stirred for five minutes to allow the formation of floc. The solution was subsequently treated by phosphatation which consists of adding 300 ppm % dry matter of milk of lime and a sufficient amount of phosphoric acid to adjust the pH to 7. The resulting mixture was stirred for an additional 30 minutes. This entire mixture was treated afterwards by micro/ultrafiltration to recover a substantially purified sugar syrup.

Microfiltration/ultrafiltration. The micro/ultrafiltration pilot plant is illustrated in Figure 1. The membranes were basically selected so that high-molecular weight substances such as dextrans, starch and colorants, could be rejected, whereas low-molecular weight compounds, such as sucrose and salts, could pass through the membrane. Four different Kerasep mineral membranes (0.2 μm , 0.1 μm , 300 kD and 15 kD) with a 0.08 m^2 filtration area supplied by Techsep (France) and capable of operating at high temperature were tested during this study.

To compare membranes performances, runs were conducted in a batch concentration mode where the retentate stream is returned to the feed tank while the permeate stream is continuously extracted, with the following operating conditions: Temperature (T) = 85°C, Transmembrane Pressure (P) = 2 bars and Velocity (v) = 5 m/s. The transmembrane pressure (P) is the average of the inlet pressure (P_i) and the outlet pressure (P_o). The cross flow velocity was attained as rapidly as possible in order to reduce the thickness of the polarization layer which forms on the surface.

Since membrane filtration system should replace both affination and clarification processes typically run on 67% solids solutions, it would be economically attractive to purify raw sugar solution above 50 Brix, despite the resulting high viscosity that generally generates low permeate fluxes. Therefore, feed solution was either a raw cane sugar solution prepared at 50 Brix or a raw cane sugar solution prepared at 50 Brix and treated by flocculation.

Permeate flux ($1/\text{h} \cdot \text{m}^2$) was periodically measured using a stop watch and a graduated cylinder.

The volumetric concentration factor (VCF) was determined by measuring the volume of permeate collected, such that:

$$\text{VCF} = 1 + \frac{V_p}{V_r}$$

where V_p represents the volume of permeate, and V_r the volume of retentate.

Each micro/ultrafiltration test was followed by an alkaline cleaning and an acid cleaning, which restored the initial water flux.

ANALYTICAL METHODS

Measurement of Brix. The Brix of the sample was measured using an Abbe refractometer.

Measurement of coloration. The coloration was measured according to the conventional ICUMSA method. The sample was diluted to 5 Brix, adjusted to pH 7, and filtered through a 0.45 μm filter. The optical density was measured at 420 nm using a spectrophotometer.

$$\text{Coloration} = \frac{\text{OD}}{C_v * l} * 1000$$

where:

OD = optical density at 420 nm

l = thickness of the absorbing solution (cm)

C_v = volumetric concentration (g/l)

The decolorization rate (%) was calculated from the coloration of the corresponding permeate and feed samples:

$$\text{Decolorization (\%)} = \left(1 - \frac{\text{Permeate Coloration}}{\text{Feed Coloration}}\right) * 100$$

In accordance with the ICUMSA method, the turbidity was expressed as the difference between the coloration before and after filtration at 0.45 μm of the sample.

RESULTS AND DISCUSSION

Effect of flocculation on the coloration of raw cane sugar solution. A series of five batch flocculation experiments was carried out using identical operating conditions in terms of flocculant dose, temperature and Brix as described above, in order to evaluate the flocculation efficiency for removing colorants. Results of initial flocculation tests are summarized in Table 1. From Table 1, it is seen that, after each treatment by flocculation, the coloration of the raw sugar liquor is considerably lowered. Indeed, the average coloration elimination reaches 32.1%. To assess the actual effect of the flocculating agent, a phosphatation (without flocculant addition) using the same quantity of lime and phosphoric acid was performed on a raw cane sugar solution. The phosphatation induces only a 20% decolorization. This latter

result confirms that the addition of the flocculant improves the efficiency of phosphatation to precipitate colorants.

Effect of flocculation on permeate flux. Four different membranes (0.2 μm , 0.1 μm , 300 kD and 15kD) were tested to determine the membrane that was the most suitable to the coupling of flocculation and micro/ultrafiltration in terms of productivity. Figure 2 illustrates the variation of permeate flux as a function of time through the selected membranes, with and without flocculation. In all eight batch filtration experiments, an initial flux drop is observed, followed by a flux stabilization. It is seen that the effect of flocculation on permeate flux depends upon the membrane pore diameter. It appears that, for the 0.2 μm membrane, permeate flux is much higher with flocculation than without. By contrast, permeate flux through the 0.1 μm membrane is lower when sugar liquor is pretreated by flocculation. These surprising results were confirmed by two other filtration tests conducted with the same operating conditions. Lastly, regarding the 300 kD and the 15 kD membranes, permeate fluxes with and without flocculation are identical. In summary, the highest final fluxes are obtained with the 300 kD and 0.2 μm membranes when flocculation is combined with micro/ultrafiltration whereas the lowest flux is logically given by the 15 kD membrane. These results suggest that flocculation is actually effective for flux improvement in the case of the 0.2 μm membrane (65 l/h.m^2 with flocculation against 48 l/h.m^2 without flocculation). A reason for this is that the particles aggregated by flocculation become too large to penetrate the 0.2 μm pores, and, in this way, may limit the infiltration of smaller particles in the pores responsible for membrane fouling. Concerning the 0.1 μm membrane, the flux is not improved by addition of flocculant. Presumably, particles that could pass through the 0.2 μm membrane may be partially retained by the 0.1 μm membrane, plugging this latter membrane, and therefore, decreasing permeate flux.

The plot of permeate flux versus volumetric concentration factor for the different membranes is displayed in Figure 3. For each run, the same trend is observed: the flux decreases sharply until VCF 2, and between this value and the final VCF, the flux becomes quite independent of VCF. As the run starts, the VCF effect on permeate flux is tied to the time effect so that one can hardly estimate how the VCF increase affects the permeate flux. Regardless of whether a flocculation was carried out or not, the flux stabilized beyond VCF 2, proving that the flocculant concentrated in the retentate loop did not modify the property of the sugar solution.

As a conclusion, it could be stated that the effect of sugar solution pretreatment by flocculation as regard flux improvement is particularly complex since this effect was found successively positive (0.2 μm membrane), negative (0.1 μm membrane) and insignificant (300 kD membrane). However, according to the experimental data, the 300 kD membrane gives the most promising results.

Effect of flocculation on permeate quality. The effect of flocculation on permeate quality is displayed in Table 2. The quality of the permeate is evaluated in terms of coloration and turbidity. Table 2 shows that, as expected, the micro/ultrafiltration process eliminates on average 90% of the turbidity, regardless of the use of flocculation. When the filtration process is preceded by a flocculation step, the permeate decolorization rate reaches on average 50% independently of the membrane used. Moreover, it is observed that filtration through 0.2 μm , 0.1 μm and 300 kD membranes induces a similar 20% permeate decolorization rate. Two important conclusions can be drawn from these observations. Firstly, the almost identical decolorization rates obtained with the three membranes (0.2 μm , 0.1 μm and 300 kD) indicate that the separation mechanism is mainly controlled by a fouling layer formed at the membrane surface by the accumulation of retained particles, rather than by the membrane itself. Secondly, flocculation pretreatment enhances considerably the color impurities retention of the membranes. Indeed, by aggregating some color molecules, the flocculating agent prevents those molecules from passing through the membrane pores. It should be noted that the flocculation test results presented in Table 1 show approximately a 32% decolorization rate of the sugar solution which is lower than the 50% obtained with the micro/ultrafiltered sugar solution. Consequently, micro/ultrafiltration retains colored molecules that were insensitive to the pretreatment. This means that combining flocculation and filtration processes results in a considerable improvement in permeate quality.

Contrary to the other membranes, without pretreatment, the 15 kD membrane removes about 40% of the colorants in the permeate, due to the tightness of its pores, which is still lower than the decolorization rate provided by the combination of flocculation and micro/ultrafiltration. The pretreatment of the raw cane sugar solution by flocculation allows to increase the decolorization to 58% on the 15 kD membrane, which corresponds to the highest decolorization rate measured with the different membranes.

CONCLUSION

The purification of raw cane sugar by flocculation and micro/ultrafiltration processes proved to be efficient in terms of turbidity removal (90%), decolorization rate (50%) and productivity (60 l/h.m²). The combination of flocculation and micro/ultrafiltration made possible the recovery of a permeate of good quality while achieving economically interesting flux performances. It was noticed that the flux decrease with the volumetric concentration factor was not significant, even at VCF 10. Meaning that at least 90% of the initial feed is economically recoverable with a 50% decolorization rate.

The membranes with larger porosities (0.2 µm and 300 kD) produced at a three-fold capacity a permeate containing less colored compounds than a tighter membrane (15 kD). The ability of the 15 kD membrane to highly decolorize at 58% the raw sugar solution pretreated by flocculation is obviously inhibited by the low capacity provided by this membrane. We believe therefore that the 300 kD membrane is the most suitable membrane for our purpose. However, the results of the experiments also indicated that the effect of flocculation on flux really depended upon the membrane pore sizes. Actually, the flocculation process applied to raw sugar containing different suspended and colloidal matters was found to be particularly complex, and therefore, must be carefully operated. Nevertheless, the removal of turbidity and the partial elimination of colorants in the permeate should facilitate the ion exchange resin decolorization process and crystallization operations. Meanwhile, the retentate should be washed (diafiltration) with water to reduce sugar loss so that the retentate purity is lower than that of molasses. Thus, retentate could be blended with the molasses.

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Table 1. Effect of flocculation treatment on the coloration of raw cane sugar solution.

Test	Coloration (ICUMSA)		Decolorization rate (%)
	Untreated syrup	Flocculated syrup	
1	3568	2398	32.8
2	3004	2161	28.1
3	3116	2267	27.2
4	3340	2118	36.6
5	3196	2048	35.9
6*	2638	2051	22.2

* In this test, the syrup was treated by phosphatation only.

Table 2. Effect of flocculation and micro/ultrafiltration on permeate quality. Operating conditions: raw cane sugar solution at 50 Brix; T=85 °C; v=5m/s; P=2 bars.

Run	Membrane Type	Flocculation	Samples	Coloration (ICUMSA)	Turbidity (ICUMSA)	Decolorization rate (%)	Turbidity removal (%)
9	0.2 µm	No	Feed permeate	3758 2888	1704 193	23.1	88.7
8	0.2 µm	Yes	Feed permeate	3340 1495	1592 144	55.2	90.9
6	0.1 µm	No	Feed permeate	3106 2389	1562 103	23.1	93.4
5	0.1 µm	Yes	Feed permeate	3004 1648	1271 142	45.1	88.8
10	300 kD	No	Feed permeate	3266 2595	1605 84	20.5	94.8
12	300 kD	Yes	Feed permeate	3196 1592	1671 130	50.2	92.2
13	15 kD	No	Feed permeate	3047 1851	1397 93	39.2	93.3
15	15 kD	Yes	Feed permeate	2579 1093	1344 72	57.6	94.6

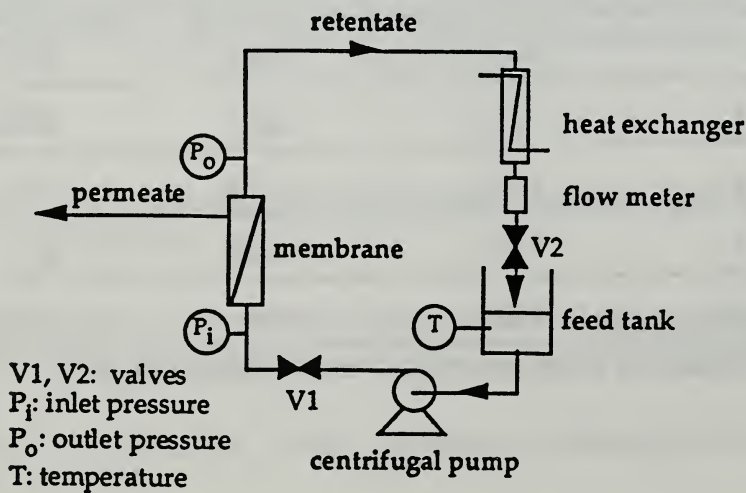


Figure 1. Schematics of the micro/ultrafiltration apparatus.

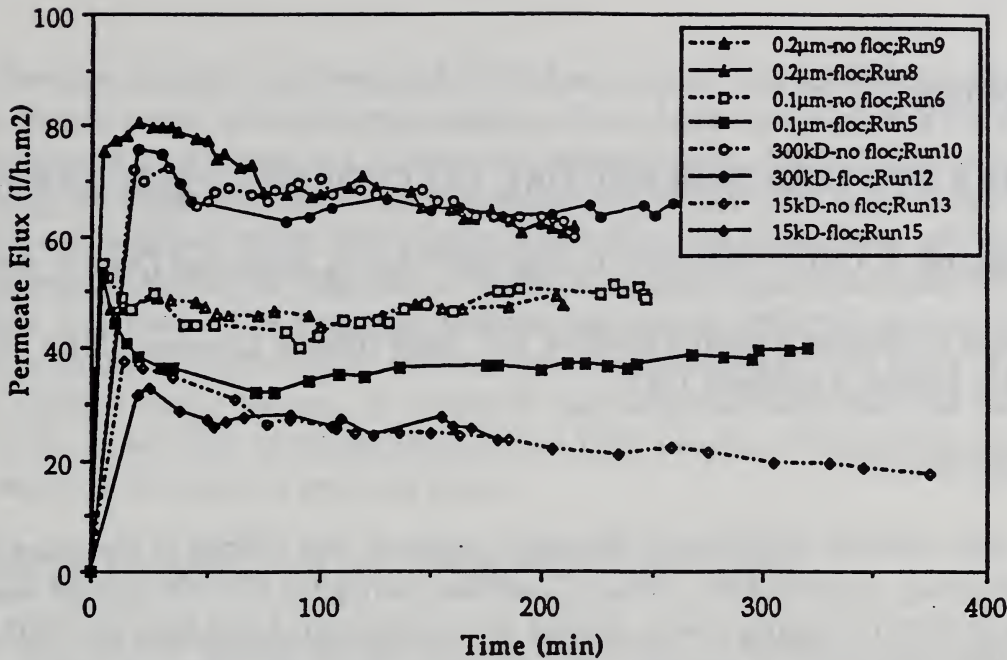


Figure 2. Variation of permeate flux as a function of time in a batch mode for different membranes with and without pretreatment by flocculation (floc). Operating conditions: raw cane sugar solution at 50 Brix; Techsep mineral membranes; T=85°C; v=5m/s; P=2 bars.

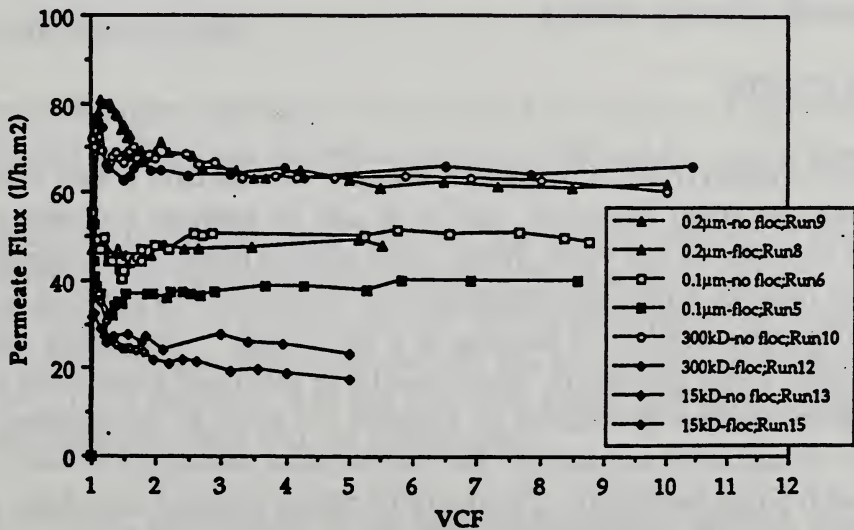


Figure 3. Variation of permeate flux as a function of volumetric concentration factor (VCF) for different membranes with and without pretreatment by flocculation (floc). Operating conditions: raw cane sugar solution at 50 Brix; Techsep mineral membranes; T=85°C; v=5m/s; P=2 bars.

POSTER

NEAR INFRARED (NIR) ROUTINE ANALYSIS OF SUGARCANE JUICES

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ABSTRACT

A near infrared NIRSystems Beverage Analyzer was utilized at the Juice Quality Laboratory, Ardoyne Farm, Houma, Louisiana, during the 1995-96 harvest season for the analyses of sugarcane juice samples of experimental clones from the USDA-ARS variety development program. The Beverage Analyzer instrument has two fixed probes so that no sample preparation was necessary. Results from 500 comparative tests for Brix and 350 tests for pol using the Beverage Analyzer and conventional methods compared favorably. The correlation coefficients for both parameters exceeded 0.97 and precision was good. This NIR method provides fast and accurate results without the use of any chemicals or clarification agents; it is, therefore, an environmentally friendly method.

INTRODUCTION

Near infrared analysis (NIR) of sugarcane juice for pol and Brix is well established (2) as a proven analytical technique, and is in use as analysis for cane payment by factories in the State of Sao Paulo, Brazil. The original method of presentation of the juice sample required further development to increase speed of analysis, to permit the analysis of a large number of samples in a short time. Initial trials used a quartz cuvette, usually 1 mm pathlength, which had to be filled and emptied by hand. Even with this labor intensive procedure, an analysis, for up to six components, takes one minute. To reduce labor and simplify the analysis, flow through cells were developed, in 1 mm and 0.5 mm pathlength. The operator places an input tube from a peristaltic pump (attached to the flow-through cell) into a container of cane juice to obtain a sample. Speed and efficiency were increased, but it was found that high mud levels in juices blocked the cell.

The Beverage Analyzer was developed for the beer industry and applied to sugarcane juice. Results on use of the Beverage Analyzer (now Juice Analyzer) over the 1995-96 sugarcane crop season in Louisiana are reported here. The NIRSystems 5665 Beverage Analyzer instrument (liquid samples only; 1100-2500 nm, transmittance mode) was installed in the Juice Quality Laboratory, Ardoyne Farm, of the U.S. Department of Agriculture, Agricultural Research Service, Sugarcane Research Unit, Houma, Louisiana. The Beverage Analyzer was run on unfiltered and unclarified juice in parallel with the regular laboratory procedures for pol (589 nm) (1) using juice clarified with a mixture of aluminum chlorohydrate, bentonite, and calcium hydroxide (3) and Brix (refractometric) (1) on 1800 samples. Results from the first few weeks of the crop are reported herein.

SENSITIVITY ANALYSIS OF LABORATORY METHODS

Four sets, of five samples in replicate but not labeled as such, were submitted to standard laboratory analysis, to determine standard lab error. Results on the sensitivity test at the Juice Quality Laboratory, Ardoyne Farm, are shown in Table 1. The coefficients of variation for both Brix and pol were excellent (less than 0.2%), reflecting the training, experience, and careful operation of technicians at the Laboratory.

BEVERAGE ANALYZER

The Beverage Analyzer (see Figure 1) can be used for analysis of liquid samples only; juices (cane and beet); syrups, molasses and liquors (diluted 1:1, as for standard tests).

Ease of operation of the Beverage Analyzer was a major reason for acceptability of this new method. The procedure for running samples was as follows: 1) the sample (60-80 ml) was placed, in a plastic beaker, on the platform of the Analyzer; 2) the two fixed probes were immersed in the sample, and the sample compartment door was closed; and 3) the spectrum was run (one key press) and after approximately 30 seconds the results for Brix and pol were printed.

Pol % juice values, read directly in the standard laboratory method, were corrected from the Schmitz equation to adjust for sucrose solids content, Brix equivalent value. Calibrations for Brix are shown in Figure 2, and for pol in Figure 3. Correlations and the standard error of calibration used for calibration and correlations and standard error of prediction used for validation are shown for Brix and pol in Table 2.

CONCLUSION

Near infrared (NIR) analysis by the Beverage Analyzer proved acceptable for analyzing large numbers of samples. Its accuracy was comparable to the standard laboratory methods for determining both Brix and pol. Precision for pol readings was similar to the Laboratory's normal precision (excellent) and in the same order of magnitude for Brix as the Laboratory's normal measurements.

The NIR Beverage Analyzer is fast. It provides rapid analysis without use of any chemicals or clarification agents thereby qualifying as an environmentally friendly method.

The NIR Beverage Analyzer requires less labor than conventional methods. One instrument operator can run 60 samples each hour, almost 500 samples in an 8 hour shift, without assistance.

Further information on analysis of the total 1800 samples run during the crop will be published in a subsequent paper.

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Table 1. Sensitivity test at the Juice Quality Laboratory, Ardoyne Farm showing the coefficients of variation for Brix and pol.

Analysis	Coefficient of variation
Brix	0.05%
pol	0.12%

Table 2. Calibration and validation of cane juice samples analyzed using standard and NIR methods.

	Calibration		Validation	
	r^2	Std. error of cal.	r^2	Std. error of prediction
Brix	0.998	0.06	0.98	0.19
pol	0.988	0.15	0.97	0.16

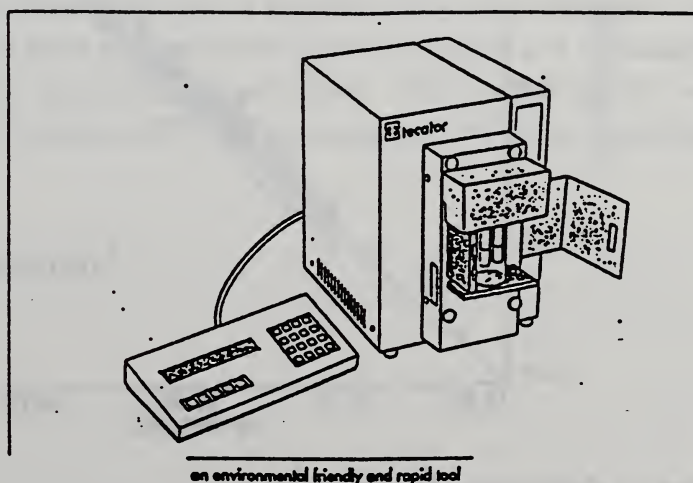
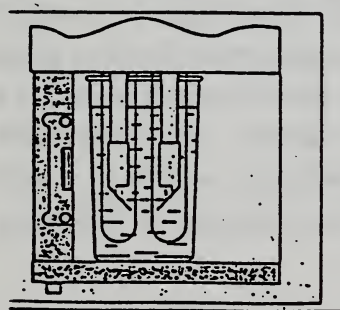


Figure 1. Beverage Analyzer - an environmentally friendly and rapid tool.

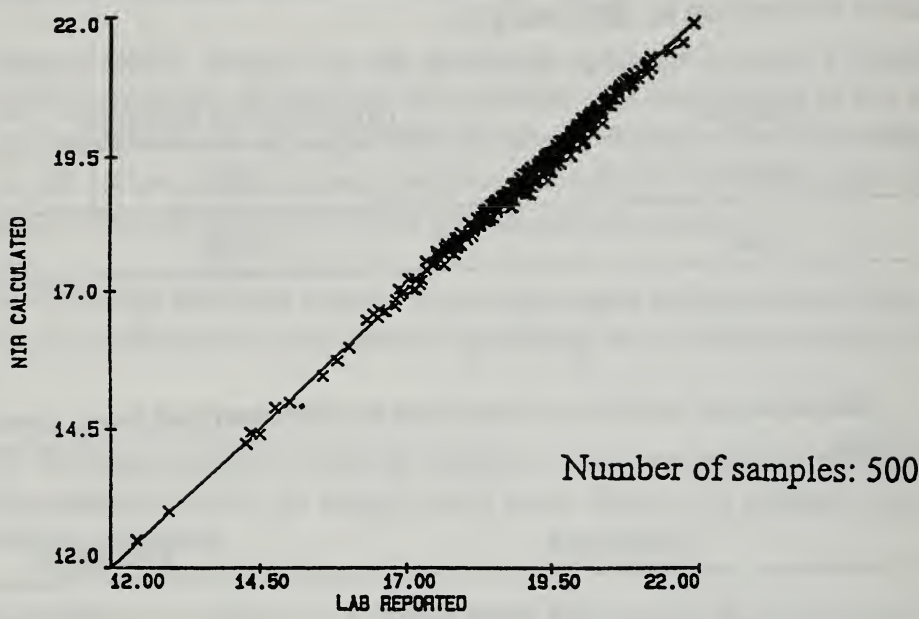


Figure 2. Calibration for Brix in cane juice.

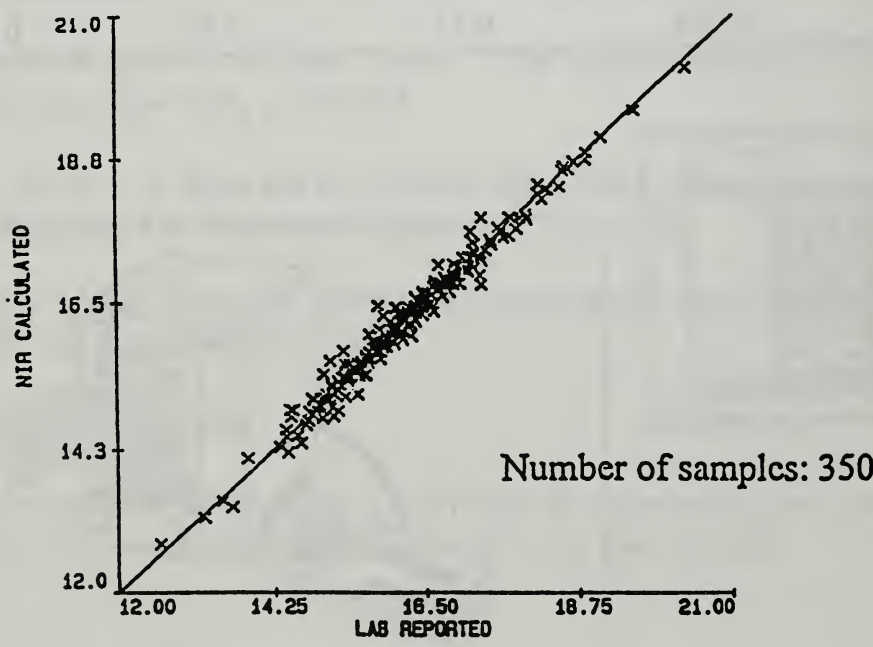


Figure 3. Calibration for pol in cane juice.

POSTER

DIFRUCTOSE DIANHYDRIDES: PRODUCTS OF SUCROSE DECOMPOSITION

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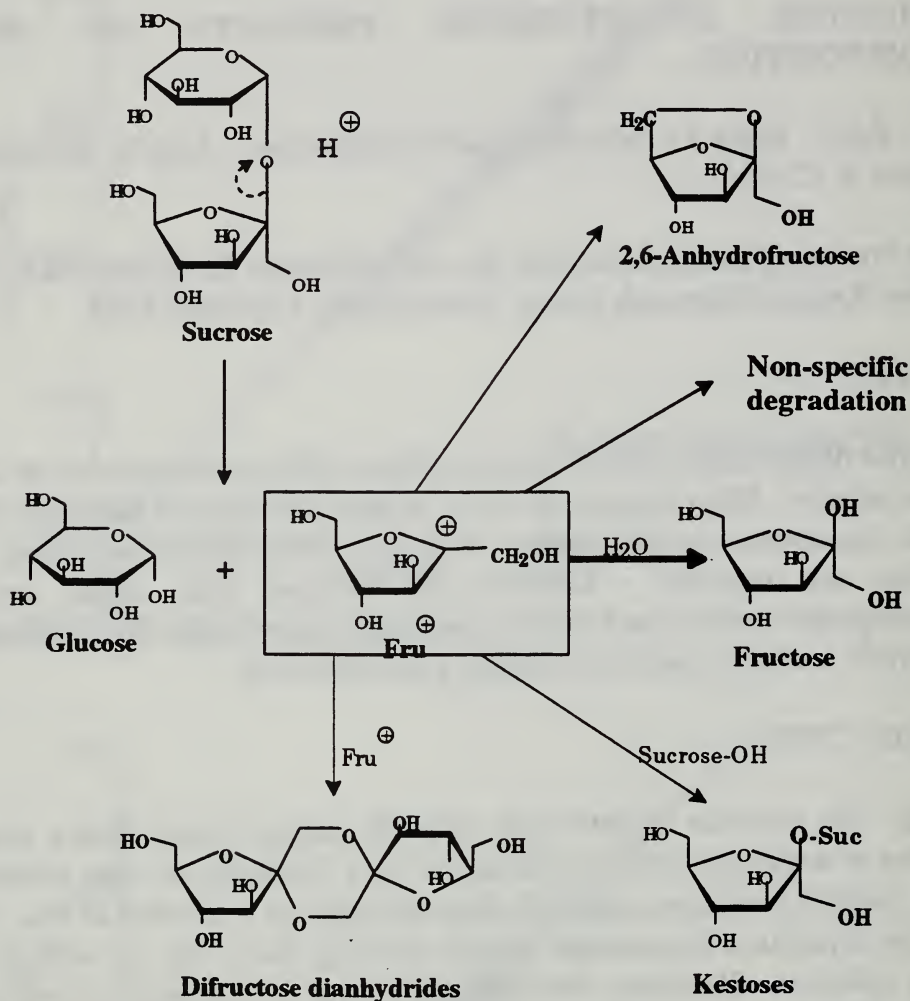
ABSTRACT

Difructose dianhydrides (DFDA's) are products of the decomposition of sucrose in aqueous solution. Many isomers can exist; at least nine isomers have been identified by mass spectrometry. Occurrence of DFDA's and conditions leading to their formation are reported. DFDA's can co-elute with sugars on several chromatographic systems and interfere with analytical results. These systems have been further analyzed; results are reported and discussed.

INTRODUCTION

Problem: The accurate measurement of small sucrose losses during the boiling processes of sugar production continues to be a challenge to sugar technologists. Several methods have been suggested; all result in only an estimation of loss. DFDA's are a class of products from sucrose hydrolysis at high Brix; they are unlikely to form in dilute solutions. Therefore, their presence in evaporator syrups and molasses are evidence of sucrose degradation during boiling processes. This poster reports "work in progress" to quantitatively measure DFDA's in evaporator syrups and molasses.

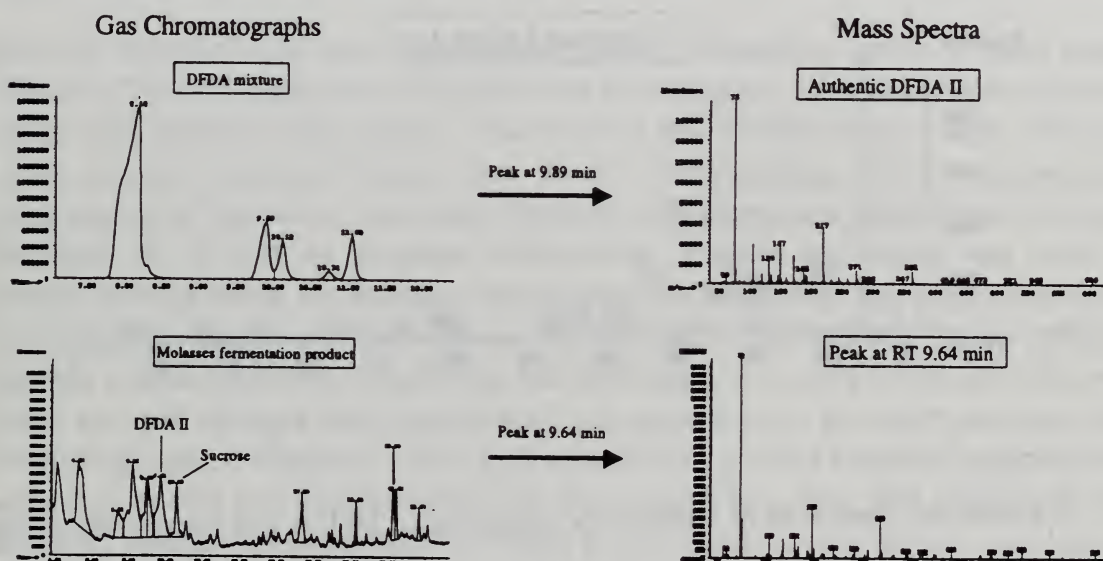
SUCROSE HYDROLYSIS OR INVERSION DURING BOILING PROCESSES.

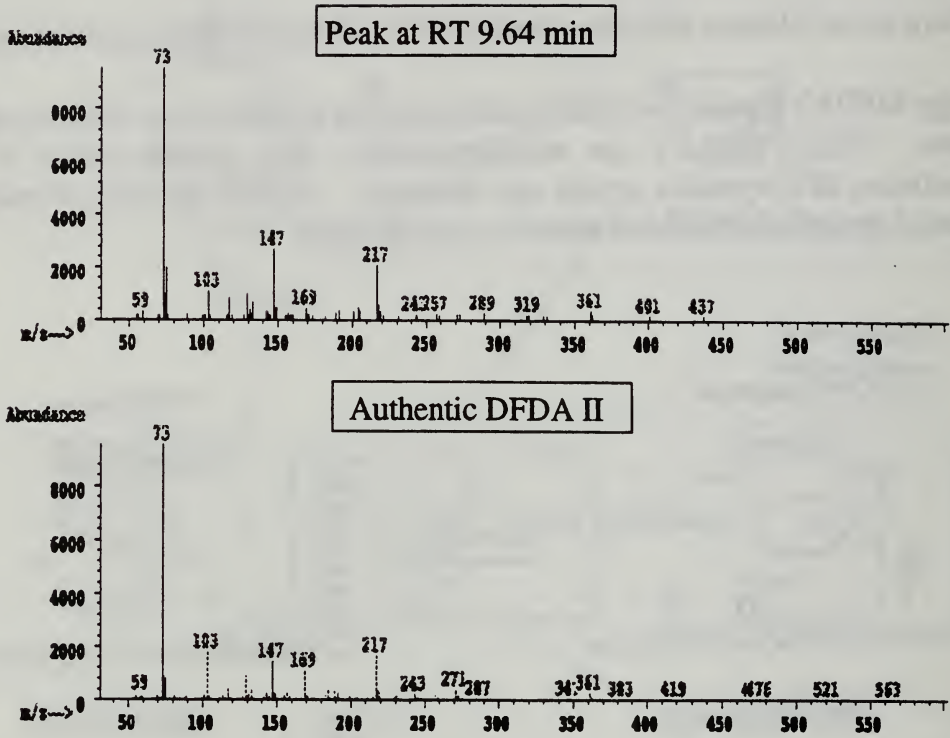


- The acid hydrolysis (inversion) of sucrose produces glucose and a fructose carboxonium ion (Fru^+).
- In dilute solution Fru^+ reacts with water to form fructose. In concentrated solutions (e.g., evaporator syrups) Fru^+ may form other products (e.g., difructose dianhydrides).
- Difructose dianhydrides form by the reaction of two fructose carboxonium ions. This event is most likely to occur during inversion at high sucrose concentrations and at high temperature.

- Their presence in evaporator syrup or molasses is an indicator of sucrose loss during the boiling processes.
- There are ca.9 known difructose dianhydrides; all have different optical rotations.

Strategy: DFDA's if present are hidden under sucrose peaks in most chromatographic systems. Since DFDA's are non-fermentable, they should persist in yeast fermentation of evaporator syrups and molasses. GC-MS analysis of completely fermented syrups and molasses should reveal DFDA's.





- We have not been able to confirm the existence of DFDA's in evaporator syrup.
- DFDA II is present in C molasses from a Louisiana cane factory.

Future work will include quantitative measurement of DFDA's via the addition of a non-fermentable mono- or disaccharide internal standard to the evaporator syrup and molasses samples prior to fermentation and the screening of other concentrated streams from sucrose manufacture for DFDA's.

POSTER

DECOMPOSITION OF SUCROSE AND FORMATION OF SUGAR DEGRADATION PRODUCTS IN REFINERY LIQUORS ACROSS TALO™-CLARIFICATION

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ABSTRACT

Sucrose chemical loss and degradation product formation across a cane sugar refinery's TALO™-clarification process were investigated. Kinetic samples of sweet water, melt (washed sugar liquor), reaction tank and clarified liquors were obtained hourly over four periods of 7 hours each. Each 7 hour sampling period was separated by an interval of one week. Ion chromatography with integrated pulsed amperometric detection (IC-IPAD), an accurate carbohydrate analysis technique, was used to monitor sucrose levels, the formation and destruction of glucose and fructose and the formation of oligosaccharides across the process. Conventional sugar refinery analysis measurements for monitoring sucrose losses, *i.e.*, pol (at 589 and 880nm), color and pH changes were determined and compared to the more accurate ion chromatography techniques. Very high correlations ($r > .95$) between polarizations measured at 589 and 880nm existed for the sweet water, melt and clarified liquor samples, whereas the correlation ($r = .868$) for the reaction tank liquors was lower, indicating that high turbidity interferes with "pol 880nm" measurements. A full statistical analysis of the data, using both correlation and analysis of variance techniques, is reported.

INTRODUCTION

Sucrose degradation not only causes chemical loss of sucrose, but also the formation of many colored and non-colored products. Such products can reduce unit process efficiencies and affect end product quality. The TALO™-clarification process has been well documented,^{1,2,3} but studies on sucrose loss across the process or across clarification processes in general^{1,4} have been limited. This study was, therefore, undertaken to measure sucrose losses across a refinery's TALO™-clarification unit

process, and compare conventional sugar refinery analyses (e.g., changes in pol) with the more accurate technique of ion chromatography with integrated pulsed amperometric detection (IC-IPAD) which can directly analyse for sucrose and invert levels. The formation of oligosaccharide degradation products across the process was also investigated, to further assess sugar loss, and from the viewpoint of identifying possible sucrose chemical loss markers.

EXPERIMENTAL

Pol. Clarification of 26g/100ml samples was undertaken by mixing with Filtercel™ and filtering through Whatman 91 paper.⁵ Clarified samples were measured at 589 and 880nm in a 20cm cell, on a Rudolph™ Autopol 880 Polarimeter calibrated in ISS (Z scale) at 20°C.

Brix. Brix was measured using a Leica Abbe Mark II™ Refractometer.

Dry Solids Content. A dry sand-vacuum oven method (GS4/7-11 [ICUMSA Methods Book 1994]) was used. The samples were dried at 64°C and measured in duplicate.

pH. Sample pH was measured at room temperature, using an Ingold™ combination pH electrode calibrated at room temperature, using standard laboratory practices.

Conductivity ash. Conductivity ash was measured on a YSI Model 32 Conductance meter using ICUMSA method GS1/3/4/7/8-13 (1994).

Color and turbidity. Color and turbidity were measured as the absorbance at 420nm (Official ICUMSA method GS1-7).

Sucrose, glucose and fructose. Diluted samples were filtered through a 0.45mm filter and sucrose, glucose and fructose concentrations were determined by IC-IPAD. Carbohydrates were separated on a Dionex™ CarboPac PA-1 column, using a NaOH gradient. See Eggleston⁶ for complete method.

Oligosaccharides. Oligosaccharide degradation products in four fold diluted, filtered samples were separated by IC-IPAD on a Dionex™ CarboPac PA-1 column, using a NaOAc gradient. See Eggleston⁶ for full method.

Model systems. Sucrose (65Brix; 100°C) was reacted under constant pH conditions using an auto-titrator. See Eggleston⁶ for complete method.

Statistical analysis. For each sample type, a separate analysis of variance was performed on sample characteristics using PROC-GLM in PC-SAS (SAS Institute, Inc., NC). The design was a one-factor randomized complete block design, where sample date was the factor and sample times were considered as replications. Means were separated using Duncan's New Multiple Range Test. For each sample type, correlation coefficients were calculated on data combined over sample dates.

RESULTS AND DISCUSSION

This study of sucrose loss across TALO™-clarification was performed on a North American cane sugar refinery. The clarifier target pH was 7.6 ± 0.1 (measured at the process temperature), the target temperature was 190°F or ~88 °C and the target brix 65.5 ± 0.2 .

Sampling

Sampling points or stages across the TALO™-clarification process are shown in Fig. 1. Samples of dilution sweet water (DSW) used to melt the washed raw sugar crystals; sugar melt (M) [or washed sugar liquor] before it was filtered and entered the reaction tank; liquor leaving the reaction tank (RTL), and clarified liquor (CL) were obtained hourly over a seven hour period. There was a 30min delay between sampling DSW and M, a 10min delay between M and RTL, and a further 30min delay between sampling RTL and CL. This seven hour sampling period was repeated four times, with a week separating each sampling period, in order to cover raw sugar and process parameter variances. All samples were immediately quenched and stored in ice to prevent further decomposition, until they were transported to and stored in a -40°C laboratory freezer. For week 1, the average pH and temperature across the clarifier was pH 7.63 and 95°C, respectively; week 2, pH 7.43 and 88.7°C; week 3, pH 7.28 and 92.7°C, and for week 4, pH 7.26 and 91.3°C.

Statistical Analysis

An analysis of potential independent variables (sample, reaction time and sampling date) and their effect on all dependent variables indicated there were no significant differences between reaction times (0-6h samples). Therefore, it is reasonable to

conclude that reaction times are truly “samples” and when they are taken during a day is irrelevant. There were significant differences between sampling dates, as the raw sugar input changed, though this variation tended to decrease across the four sampling stages (see Fig. 1). By the final clarification stage (CL samples), significant differences ($p < 0.05$) between sample dates were found only for turbidity, pH, color, pH and color change (from the reaction tank to the clarifier) measurements. Purity₅₈₉ and 880nm values were the most stable variables across sample dates and across the TALO™-clarification process.

Conventional Refinery Analyses

Conventional refinery analyses included pol and purity (at 589 and 880nm), brix, dry solids, turbidity, color and conductivity ash measurements (see Eggleston et al⁷ for full graphical results).

Very high correlations ($r > .953$) between polarizations measured at 589 and 880nm existed for the DSW, M and CL samples, whereas the correlation ($r = .868$) for the RTL samples was lower, indicating that turbidity interferes with “pol 880nm” measurements. Wilson⁸ recently reported that certain non-sucrose compounds, including dextrans, impact the pol 880nm measurements. The correlations between color (ICU_{420nm}) and pH were unexpectedly negative. Lower pH values would have been expected to produce less color,³ as more color is formed at higher pHs, mostly due to the alkaline degradation of monosaccharides. The likely explanation for the negative correlations is the overriding effect of phosphoric acid additions. This also explains the pH drop from the reaction tank to the clarifier. Over the whole study, the average turbidity removal across the clarifier was a very good 96.54%. Average color removal was 37.99%, ranging from 26.25% in week 1 to 44.84% in week 4. Generally, the lower the pH (as measured in the refinery and the laboratory) the higher was color removal. This agrees with the observations of other workers⁹ that “color removal is proportional to the amount of phosphoric acid (P₂O₅) added”, although of course lime needs to be added to maintain pH and prevent inversion from addition of phosphoric acid alone.

Markedly higher color and ash values were found in the DSW samples, which strongly suggests that these non-sugar compounds are being recycled back into the melter; this confirms the previous observations of James et al.¹⁰

Sucrose Decomposition and Invert Formation

As the statistical analysis of the conventional refinery data indicated that significant variation exists only between the 4 different process samples and sampling dates, selected reaction time composites of the 4 different sample stages were analysed for sucrose and invert (glucose and fructose) levels by IC-IPAD.

It was expected that fructose and glucose would be formed during the phosphatation defecation process, but they were apparently degraded there as well. Similar observations were noted by Clarke and Brannan.¹¹ Although further studies using IC-IPAD are to be undertaken on all the samples, an initial direct comparison of results with corresponding $\text{pol}_{589\text{nm}}$ values, suggests that pol values underestimated or did not detect the sucrose losses as measured by IC-IPAD. This is to be expected, because although sucrose hydrolysis (acid degradation) can decrease the pol value (and the purity), the subsequent degradation of fructose will result in an increased pol value and an underestimation of sucrose loss.¹²

Oligosaccharide Degradation Products: Possible Sucrose Loss Markers

The most accurate determination of sucrose loss in sugar refining would be to analyse for a stable degradation product, i.e., a marker compound. Oligosaccharides are formed in the breakdown of sucrose and monosaccharides, under acid and alkaline conditions, and have strong potential as possible stable markers. An IC-IPAD method, using a strong NaOAc gradient, was developed to separate oligosaccharides (up to 12 degrees of polymerization) in concentrated sugar solutions commonly found in the refinery (see Eggleston⁶). Fig. 1 illustrates typical "fingerprint" oligosaccharide chromatograms of composite DSW, M, RTL and CL samples from week 4. The four sample chromatograms were very similar and it is obvious that the sample "fingerprint" oligosaccharides were, at least partially, determined by the melt (or raw sugar) origin. The DSW samples have more and larger oligosaccharide peaks, suggesting further degradation occurred in the mud desweetening system, reflecting the observation that the highest color was found in the DSW samples. A predominant peak, denoted B, was visible in all the samples.

Fig. 1 also illustrates a comparison of DSW, M, RTL and CL oligosaccharide chromatograms (from week 4) to those of model 65 Brix pure sucrose degraded samples, reacted at 100°C for 1 and 8h.⁶ The model samples were reacted under constant pH 7.5 and 8.3 conditions,⁶ similar to those found in the TALO™-clarifier.

It was highly interesting to note that several similar peaks (A, B, C, D and E) were present in all the refinery and model samples, and these warrant further investigation, i.e., as identification of possible markers.

SUMMARY AND CONCLUSIONS

Clarification

- * major factors affecting color removal in TALO™-clarification are pH and level of phosphoric acid addition
- * non-sugar compounds, including color, are being produced in the mud de-sweetening system and then being recycled back into the melter
- * invert sugars are being simultaneously formed and degraded during the phosphorylation process
- * losses in refinery clarification can be measured satisfactorily with IC-IPAD

Measurement Systems

- * turbidity components interfere with "pol 880nm" measurements
- * pol values underestimated or did not detect the sucrose losses as measured by IC-IPAD

Marker Compounds

- * numerous oligosaccharide IC-IPAD peaks are present in the samples and, at least partially, reflect the melt or raw sugar origin
- * several similar oligosaccharide IC-IPAD peaks were present in the refinery and model sucrose degraded samples and, therefore, show strong potential as sucrose loss markers

One of the major aims of this project is to identify stable marker compound(s) in different unit processes in the sugar industry, including the TALO™-clarification unit process in this study, to accurately and easily determine actual chemical sucrose loss.

Further identification of the oligosaccharide degradation products, discussed in this study, is currently being undertaken.

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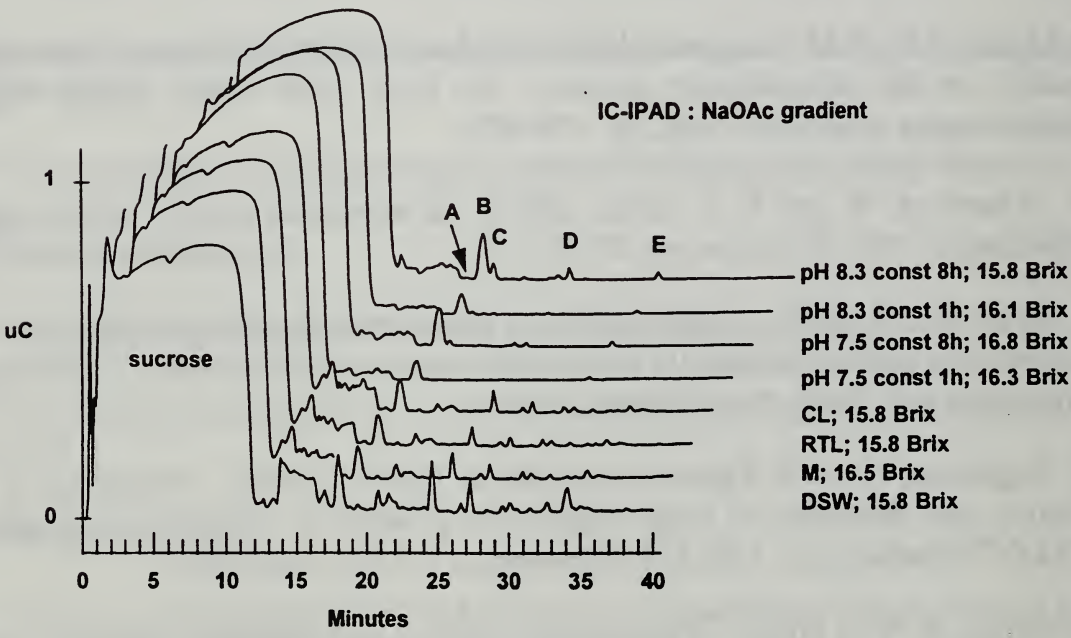


Figure 1. Comparison of "fingerprint" oligosaccharides present in composite refinery samples (wk 4) with those in model sucrose samples (65 Brix), degraded at 100C for 1 and 8 h, under similar pH constant conditions.

POSTER

STARCH, POLYSACCHARIDE AND PROANTHOCYANIDIN IN LOUISIANA SUGARCANE VARIETIES

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INTRODUCTION

The sugarcane breeding program in Louisiana selects new varieties for at least 24 characteristics, including maturity, sucrose content, purity, harvestability, fiber content, tons of cane per unit area, and resistance to selected diseases and insect pests. These factors contribute to the development of cane varieties that are well adapted to Louisiana growing conditions, while producing the maximum possible yield of sugar per unit area.

Other components, present in low concentration, influence the quality of cane juice and yield of sugar in processing, but these are not normally selected for or against in the breeding and quality selection program. Starch and other cane polysaccharides are examples of such significant minor components.

Soluble polysaccharides expressed into juice include:

Starch, a storage polysaccharide of sugarcane,
Phytoglucan (Roberts, *et al.*, 1985),
Arabinogalactans (Roberts, *et al.*, 1976),
Other soluble cell wall polysaccharides (Clarke, *et al.* 1988)

Microbial polysaccharides that can be formed on cane, most commonly, dextran

Polysaccharides lower the quality of juice and the raw sugar in several ways:

1. During processing: Increase viscosity; slow or inhibit crystallization; increase the loss of sucrose to molasses (ie, have a high melassigenic effect).

2. Difficult to remove in process; tend to be included in the raw sugar crystal; go into the refining process; cause similar problems in refining.
3. May contribute to polarization distortion, especially in polarization methods that do not use lead clarification.

In Louisiana, sugarcane is immature when harvested because of the short seven to nine month growing season. Starch concentration is elevated in immature and maturing cane (Balch, 1953). Raw sugar produced early in the crop season in Louisiana can be high in starch (Godshall, *et al.*, 1990), reflecting the high starch in the cane juice.

OBJECTIVES

- (1) To determine if sugarcane varieties differ in content of total polysaccharide (TPS) and starch.
- (2) To determine if the concentration of these changed significantly during the harvest season.
- (3) To measure proanthocyanidin associated with soluble cell-wall polysaccharides.

Because of the wide genetic potential in sugarcane, this information could be helpful to breeders and may assist growers in choosing varieties that produce higher quality raw sugar.

MATERIALS AND METHODS

Varieties tested: Ten sugarcane varieties developed for Louisiana conditions.

Sampling dates: Oct. 1, Oct. 15, Nov. 13, and Dec. 10, 1990

Sampling Procedure: Fifteen stalks of each variety were randomly selected on each sampling date from each of four replicates. Varieties planted in 3-row plots (1.7 m wide by 12 m long), and arranged in a randomized complete block design. Stalks topped approximately 10 cm below the apical meristem, weighed, and milled once through a 3-roller mill with 50-55% juice extraction.

Starch: Juice was preheated just to boiling to inactivate starch-degrading enzymes. Starch was determined using iodine colorimetry according to the method of the South African Sugar Milling Research Institute (SMRI) (Chen, 1985), using the filtration modification reported in 1990 (Godshall, *et al.*, 1990). Starch was reported as ppm on cane juice solids.

Total polysaccharide: Measured colorimetrically using phenol-sulfuric acid after precipitation and isolation from sugarcane juice with 80% alcohol, using the SPRI method (Roberts, 1981). TPS reported as ppm on cane juice solids. Before precipitation of the polysaccharide, the cane juice was filtered on a coarse filter paper, to remove insoluble starch granules and bits of cellulosic material, which would interfere in the test.

Anthocyanidin bound to polysaccharide: Determined by absorption at 485 nm after conversion of the colorless precursor to the colored anthocyanidin moiety, while conducting the TPS test in the following manner: After polysaccharide was isolated as the 80% ethanolic precipitate during the TPS test, it was boiled briefly with 1% sulfuric acid. The sulfuric acid treatment converts the colorless anthocyanidin precursor to the pigmented anthocyanidin form. The sample was filtered on filter paper (S&S No. 588) and absorbance of the filtrate measured. Only anthocyanidin associated with polysaccharide was measured by this test. Results are reported as absorbance x 1000 on cane juice.

Other testing dates: Seasonal means of selected varieties were measured in 1991, 1992, and 1993 to determine if relative concentrations in varieties remained the same over time.

RESULTS

Starch: Figure 1 shows the 1990 mean starch concentrations for sugarcane varieties, including statistical groupings. Varieties with different letters are significantly different at the 95% confidence level. The mean varietal value for starch for all varieties combined was 670 ppm for the season. Starch showed a 5.3-fold increase from the highest variety to the lowest variety. Changes within varieties for harvest dates were much smaller.

Total polysaccharide (TPS): Figure 2 shows the 1990 mean TPS concentrations for sugarcane varieties, including statistical groupings. Varieties with different letters are

significantly different at the 95% confidence level. The mean varietal value for TPS for all varieties combined was 2,139 ppm for the season. TPS showed a 2.2-fold increase from the highest to the lowest variety. Changes within varieties for harvest dates were much smaller.

Anthocyanidin: Figure 3 shows the 1990 mean anthocyanidin values for sugarcane varieties, including statistical groupings. Varieties with different letters are significantly different at the 95% confidence level. The over-all mean anthocyanidin value for all varieties combined was 32 absorbance units for the season. Anthocyanidin showed a 6.5-fold increase from the highest to the lowest variety. Changes within varieties for harvest dates were much smaller.

Seasonal Trends: No major seasonal trends were noted in the three components over the season within varieties: Some varieties increased, some decreased and some remained unchanged, but changes tended to be small. Weather conditions had more of an effect (see below).

Varietal Differences: Differences among varieties were much more significant than changes that occurred during the growing season. Figure 4 compares starch in the highest and lowest varieties, CP 72-370 and CP 70-321 on the four harvest dates. Figure 5 compares TPS in the same two varieties. The TPS peak in November is attributed to unseasonably warm, wet weather, which caused new growth.

Stability of Concentrations: During the subsequent years, as part of ongoing studies in the varietal development program, starch, polysaccharide and proanthocyanidin were measured. Results showed that varietal concentrations remained in the same range over time. Figure 6 shows seasonal mean concentrations of starch for selected varieties. Figure 7 shows seasonal mean concentrations of TPS for several varieties. The 1990 seasonal means are included for ease of comparison. The TPS results from a 1989 study are also included (Legendre, *et al.*, 1991). Figure 8 shows the anthocyanidin results for 1992 compared to 1990. The data are shown respectively in Tables 1, 2 and 3.

Effect of Polado: The effect of the growth regulator, Polado, on starch, TPS and anthocyanidin was determined for 3 varieties in 1992 (Legendre, *et al.*, 1994): CP70-321, CP72-370, CP65-357. Starch increased in CP 70-321 from a varietal mean of 250 ppm to 466 ppm, but did not change in the other two varieties. TPS increased

significantly in CP72-370 but not in CP70-321 or CP 65-357. Anthocyanidin increased 62% in CP72-370 and 40% in CP65-357 but had no effect on CP 70-321.

ANTHOCYANIDIN PIGMENTS

As part of SPRI's continuing studies on the polysaccharides of the sugarcane plant, it was noted that isolated sugarcane polysaccharide, when treated with hot, dilute mineral acid, developed red pigmentation. This indicated the presence of proanthocyanidin (colorless precursor of anthocyanidin pigment), possibly bound to the polysaccharide. A subsequent study showed the proanthocyanidin to be associated with cane polysaccharide (Godshall and Grimm, 1994). The sugarcane plant is known to produce copious amounts of anthocyanidin pigments in response to numerous stresses, including disease (Hokama, 1973; Messiaen and Quoit, 1969), and these pigments may have some biological activity. Anthocyanidin can be induced by cold temperatures or intense sunlight. Healthy, unstressed sugarcane juice and sugarcane tissues do not normally contain any red pigmentation, but a red color is induced in some tissues and some varieties by treatment with hot mineral acid. The test for proanthocyanidin described in this study measured only polysaccharide-associated pigment.

The structure of the colorless anthocyanidin precursor, whether "leucoanthocyanidin" or "proanthocyanidin" in the sugarcane was not determined by this study. Proanthocyanidin refers to condensed tannins or oligomeric flavan-3-ols, which can be depolymerized in acid solution to anthocyanidins, while leucoanthocyanidin is the name given to monomeric forms that produce anthocyanidins on heating with acid (Watterson and Butler, 1983; Harborne, 1966). Luteolinidin, a 3-deoxyanthocyanidin, has been identified as the main red-orange pigment in canes infected with red rot disease (Messiaen and Quoit, 1969; Godshall, 1984). Some of the spectral characteristics of the pigment formed in this study are similar to those of luteolinidin. Because the anthocyanidin pigment is freed from the polysaccharide by hydrolysis with acid, it is possible that these exist as the monomeric colorless form covalently bound to polysaccharide.

Figure 9 shows the structure of the proanthocyanidin, luteoforol, which is light yellow. When treated with acid, it converts to the orange-red 3-deoxyanthocyanidin, luteolinidin, which has been identified as the major red pigment in sugarcane (Messiaen and Quoit, 1969).

CONCLUSIONS

- (1) Sugarcane varieties differ significantly in the concentration of starch, TPS, and anthocyanidin in juice. Concentration differences range as high as 6.5 times for anthocyanidin, 5 times for starch, and 2 times for TPS, from the variety with the highest concentration to that with the lowest concentration.
- (2) The two most important commercial varieties in Louisiana, CP72-370 and CP70-321 present strong contrasts. CP72-370 is highest, by far, in the three components studied, and CP 70-321 is among the lowest (Refer to Figures 4 and 5). A previous study had shown that around 30% of the juice starch will end up in the raw sugar crystal (Godshall, *et al.*, 1990). At about the 200-250 ppm starch level in raw sugar, the refinery may begin to experience process difficulties (Hidi and McCowage, 1986; Devereux and Clarke, 1986). It is, therefore, useful to know which varieties are very high in starch so that their use can either be minimized or the raw sugar produced from them can be mixed with sugar made from low starch varieties. Alternately, the mill could be prepared to add alpha-amylase in the process (evaporators) to remove the starch.
- (3) Varietal differences are considerably more significant than changes that take place during the harvest within varieties, due to harvest time.
- (4) While sugarcane juice composition is sensitive to weather changes during the harvest (i.e. unseasonably warm and wet periods), varietal differences are more significant than seasonal or weather changes. However, increases in the concentration of starch and TPS can be expected because of hot or wet weather during the harvest.
- (5) The concentrations of starch, TPS and proanthocyanidin in cane varieties are consistent from year to year.

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Table 1. Comparison of seasonal concentration of TPS in selected sugarcane varieties.

Variety	TPS, ppm on Cane Juice Solids			
	1989	1990	1991	1992
CP 72-370	4804	3234	3308	4957
CP 79-318	3562	2577	3082	3126
LCP 82-89	not available	2161	3107	2841
CP 65-357	3216	2128	2849	2908
CP 74-383	not available	1931	3162	2671
CP 70-321	2553	1455	2496	2862

Table 2. Comparison of seasonal concentration of starch in selected sugarcane varieties.

Variety	Starch, ppm on Cane Juice Solids		
	1990	1992	1993
CP 72-370	1460	1867	1548
CP 79-318	986	1092	986
LCP 82-89	566	701	538
CP 65-357	506	745	557
CP 74-383	611	590	636
CP 70-321	275	239	220

Table 3. Comparison of seasonal concentration of proanthocyanidin in selected sugar-cane varieties.

Variety	Proanthocyanidin, absorbance on cane juice	
	1990	1992
CP 72-370	65	74
CP 76-331	59	41
CP 65-357	40	37
LCP 82-89	22	25
CP 79-318	20	15
CP 70-321	15	21
CP 74-383	10	12

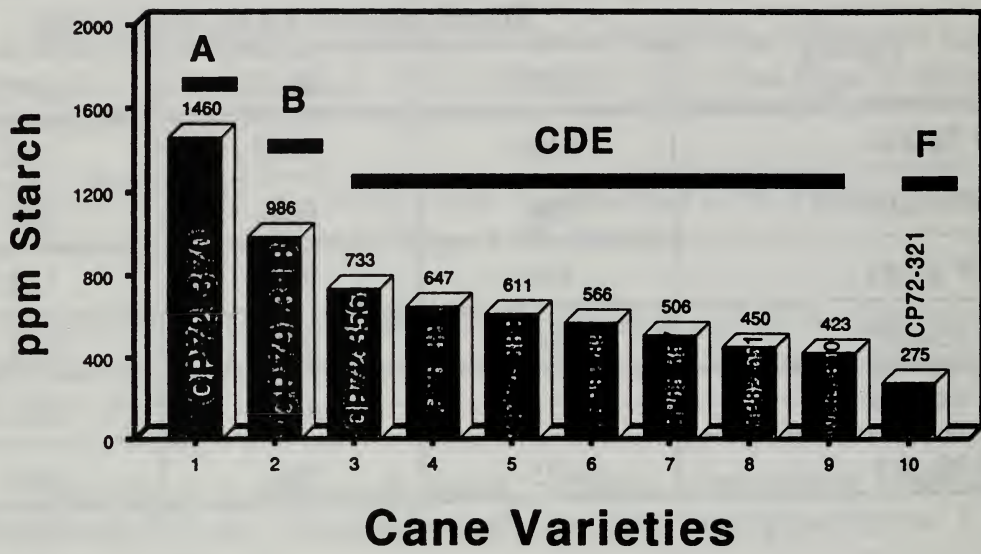


Figure 1. Starch in cane varieties. Seasonal means and statistical groups.

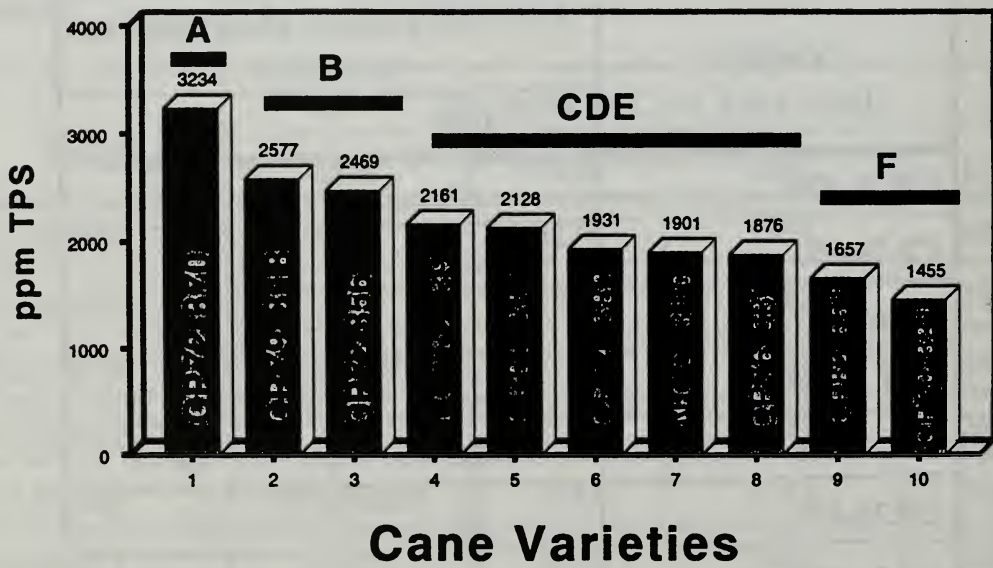


Figure 2. Total polysaccharides in cane. Seasonal means and statistical groups.

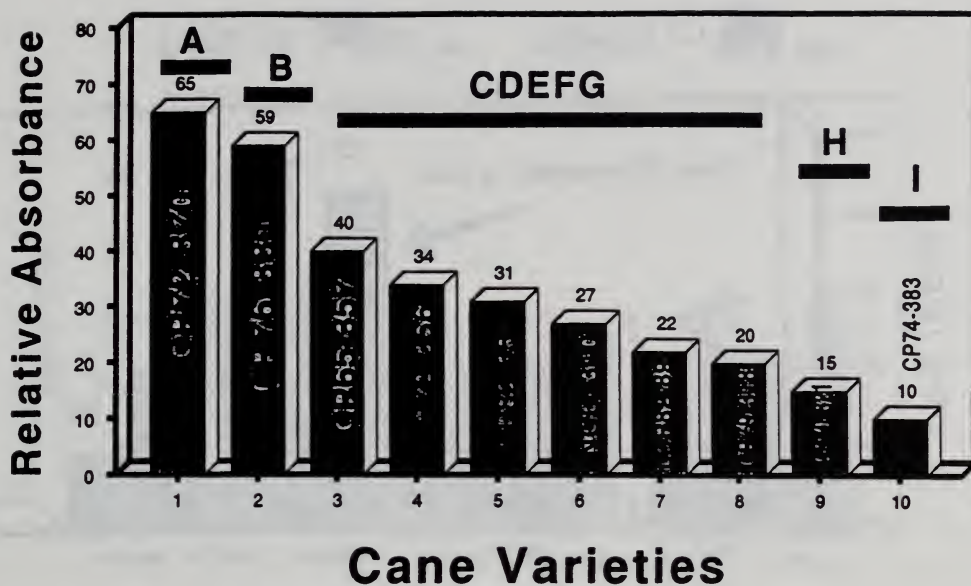


Figure 3. Proanthocyanidins in cane. Seasonal means and statistical groups.

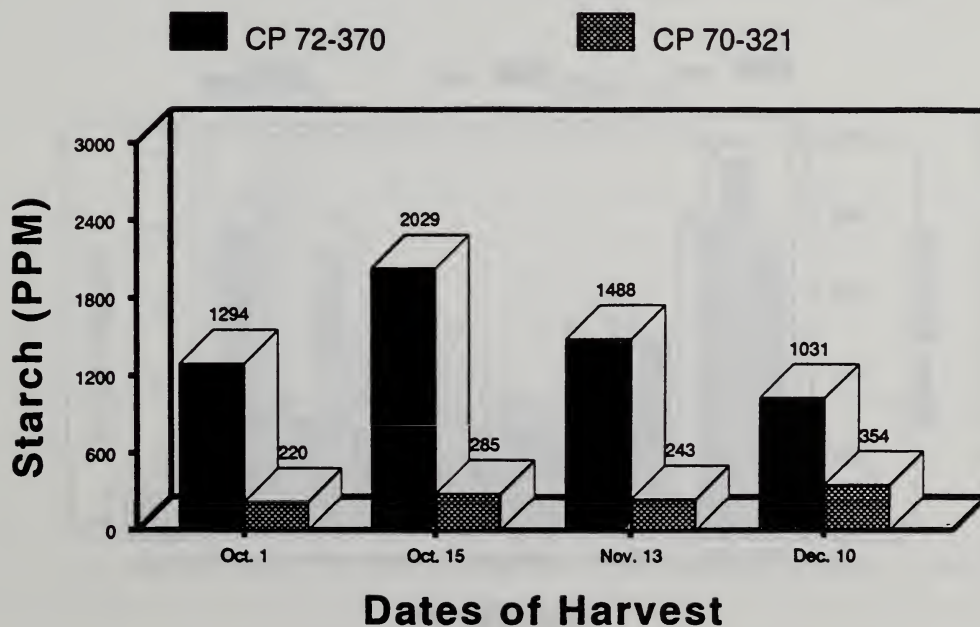


Figure 4. Comparison of starch in two varieties.

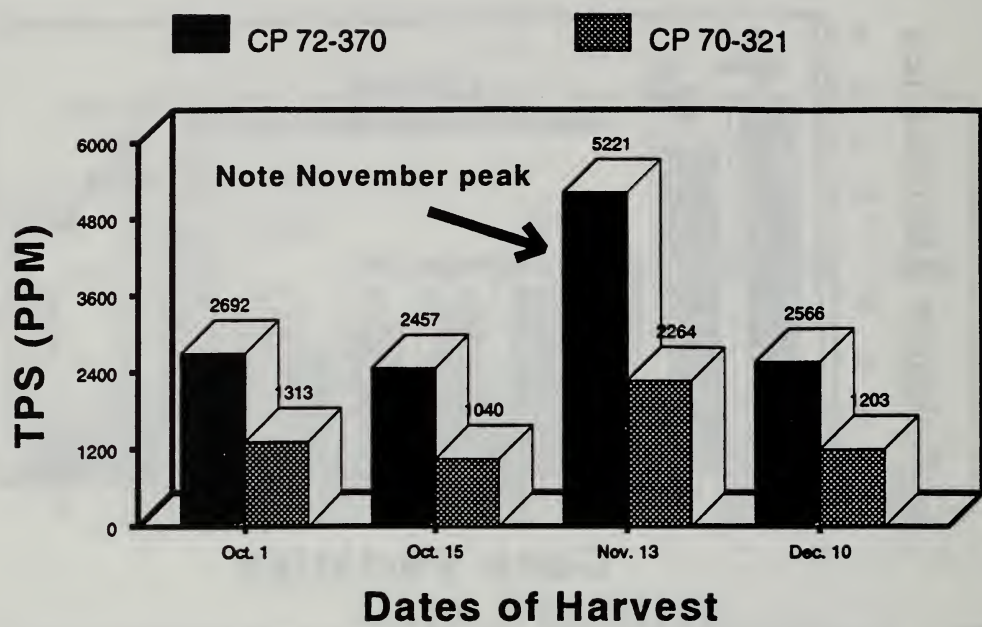


Figure 5. TPS in two varieties.

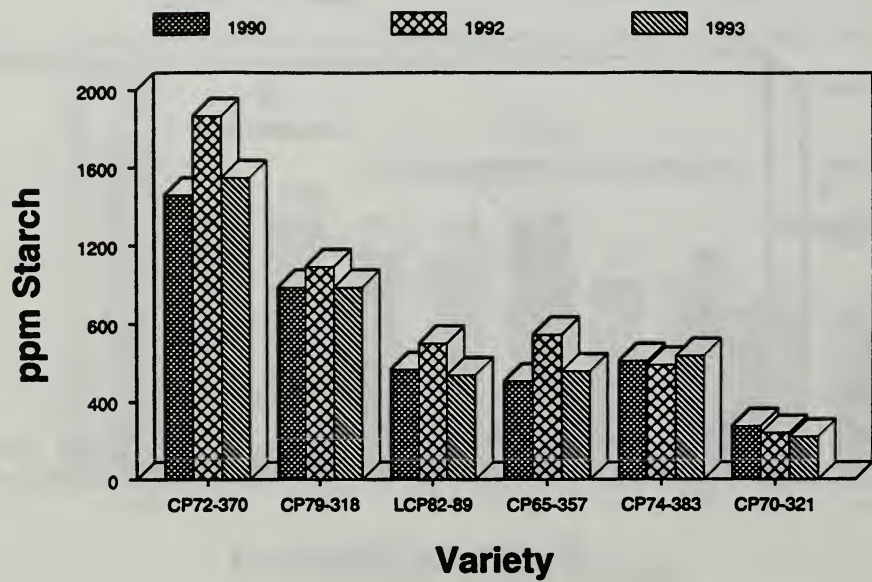


Figure 6. Starch in cane varieties. Annual varietal means.

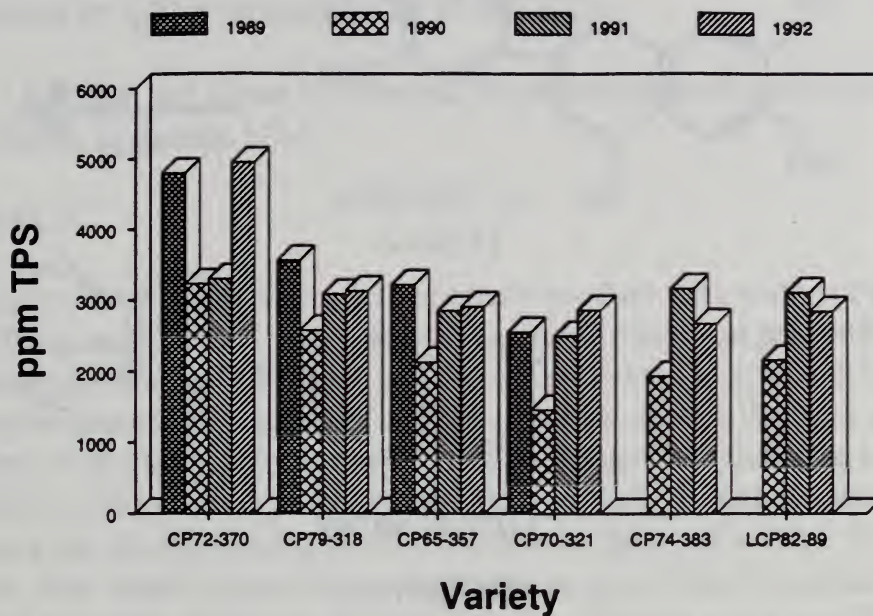


Figure 7. TPS in cane varieties. Annual varietal means.

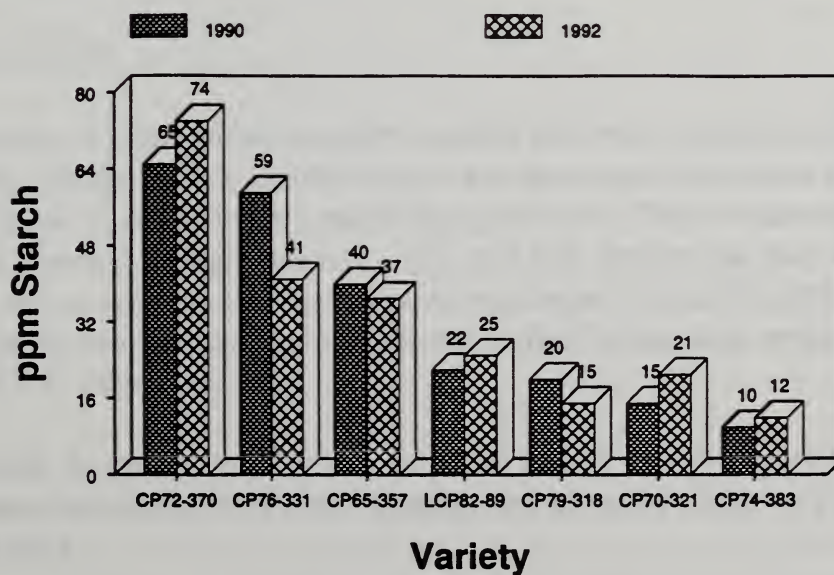


Figure 8. Anthocyanidin in cane varieties. Annual varietal means.

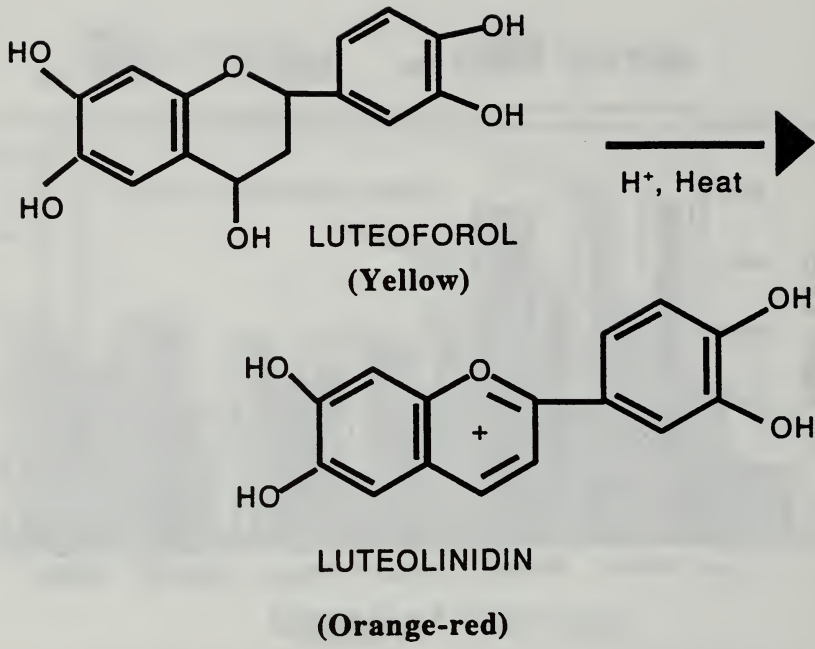


Figure 9. Structures of some proanthocyanidins.

POSTER

CHANGES IN SOFT SUGARS ON STORAGE

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ABSTRACT

Soft sugars represent a value-added specialty product with wide popularity in the United States and Canada. Their desirable taste and functional properties contribute to widespread use in confectionery and bakery products. They are characterized by a small grain size and by high moisture, color and ash, factors that may contribute to instability on storage. In this study, increases in color and titratable acidity in soft sugars from various sources were monitored over time. A high moisture content in the sugars was the best predictor of an increase in color and acidity. It was evident, however, that other factors also contributed, as not all high moisture soft sugars exhibited the same degree of storage changes. Organic and phenolic acids were monitored in selected sugars to determine if these components contributed to storage changes. Extraction methods for isolating organic and phenolic acids from soft sugars are described. A large number of acids was identified by gas chromatography and mass spectrometry.

INTRODUCTION

Soft sugars are a value-added specialty product with wide popularity in the United States and Canada. Their desirable taste and functional properties contribute to widespread use in confectionery and bakery products. They are characterized by a small grain size and by high moisture, color and ash, factors that may contribute to instability on storage, reflected by increases in color and titratable acidity over time. An increase in acidity may cause problems in food mixes that contain leavening agents, such as cake mixes.

In this study, increases in color and titratable acidity in soft sugars from various sources were monitored over time. Organic and phenolic acids were measured in selected sugars.

METHODS

Buffering capacity test: 50 g sugar + 200 ml water; titrate with 0.1 N NaOH (standardized) to pH 7.0. Reported as ml NaOH/100 g sugar. (Method received from a sponsoring company.)

Color: ICUMSA Method GS1-7.

Moisture: ICUMSA Method GS2/1/3-27.

Heated Color Ratio: Ratio of color of sugar before and after the test for moisture, which heats the sugar for 3 hrs at 105°C.

Extraction of acids: Acids were extracted from soft sugars using a strong anion exchange micro cartridge. Three g sugar dissolved in 20 ml H₂O was pre-filtered on analytical filter aid, and passed through strong anion extraction micro cartridges (SAX-Alltech, 600 mg). Sugar was washed off by passing water through the cartridge. The retained compounds were desorbed with 3 ml 3N formic acid, which was evaporated off to prepare the extract for gas chromatography, as the trimethylsilyl derivatives. Extractions were performed at 4 months and 14 months.

Extraction of phenolics: A surface extraction method using methanol was used to extract phenolics on the surface of the sugar crystals. 25 g sugar was stirred overnight with 100 ml methanol. The resulting methanolic extract was subjected to rotary evaporation under reduced pressure to remove methanol and water. The syrupy residue was redissolved in water, acidified with HCL, and extracted with ethyl acetate. Ethyl acetate was removed by rotary evaporation and the extract prepared to gas chromatography as the trimethylsilyl derivative. Extractions of control sugar and lower moisture sugar were performed at 14 months only.

GC/MS: Compounds were identified as trimethylsilyl (TMS) derivatives using an HP5890A GC and HP5972A Mass Selective Detector.

RESULTS

Figure 1 shows the color development in 5 soft sugars (top graph) and the development of titratable acidity in the same sugars (bottom graph). The development

of titratable acidity appeared to plateau after 100-120 days, but color continued to increase slowly at a linear rate.

Figure 2 shows the effect of moisture on the percentage of color increase after 4-6 months of storage (top graph). The bottom graph shows the correlation of the heated color ratio and the percentage of color formation. Color increase correlated moderately well with moisture content ($R^2 = 0.81$). The heated color ratio had less correlation ($R^2 = 0.65$), but may be an indicator of the potential for color to increase on storage in some soft sugars.

Figure 3 shows color and acidity formation in four sets of sugars, in which subsamples were dried to two lower moisture levels. After 7 months, the acidity in the high moisture controls was notably higher than in the low-moisture samples, but there was less effect on color. The final color of the low moisture samples was lower than the high moisture control. However, color had increased significantly in all of the sugars over the 7 months. The quality of the color of the high moisture controls was different after 7 months, than the low moisture sugars, having a redder hue. This difference is not reflected in the ICUMSA color measurement.

Figure 4 shows the color and acidity increase for a soft sugar at 3 moisture levels up to 145 days; this sugar is also shown as part of the set of sugars in Figure 3 and is the one used for the extractions in Table 2.

Equations for color increase were developed for soft sugar samples from 7 refineries. These equations are summarized in Table 1. All the equations for all the samples from all the refineries showed a strong linear correlation between color and age (in days) of the sugar. This would suggest that soft sugars normally have a tendency to increase in color on storage, but that the rate of increase (the slope of the line) is different for individual refineries, and possibly for the type of sugar (whether it is light or dark in color to start). The data suggest that some refineries (ie, B and C) may tend to produce sugars that increase in color at a faster rate than others.

Table 2 shows the concentration of organic acids in one soft sugar (Y5018, Figures 3 and 4) at two moisture levels at 4 and 14 months, and phenolic acids at 14 months. The results show no differences in the concentration of any of the phenolic acids at the two moisture levels at 14 months. Since data are not available for phenolics concentrations from an earlier date, it is not possible to know if their concentration had gone down in both samples, contributing to the color increase that did occur.

Quinic and malic acids both originate from the sugarcane plant, and showed no change over time at either moisture level. The remaining compounds (lactic acid, glyceric acid, erythronic acid, gluconic acid, and xylonic acid lactone) are related to sugar degradation in process. Lactic acid and the unidentified acid at 5.39 min showed the greatest difference with time at the different moisture levels.

OBSERVATIONS AND CONCLUSIONS

1. The data in this study showed that many, if not most, soft sugars increase in color and titratable acidity with time, but rates of change can be quite different. This has implications for formulated foods which contain a mixture of a leavening agent and soft sugar, as the acidity may increase enough with time to inactivate the leavening agent.
2. Titratable acidity increases stabilized (stopped increasing) around 100-120 days after production.
3. Color increase did not show a corresponding plateau, but continued to increase at a linear rate. Color increase was quite significant in some sugars, as much as 50% within 6-8 months.
4. Moisture is a good predictor of color and acidity increase on storage, but obviously is not the only cause, as some high moisture soft sugars did not fit the prediction.
5. The ratio of color formed on heating a soft sugar may also indicate its tendency to form color on storage. A heated color ratio >3.5 showed some correlation with higher color formation. This is not a definitive test, however.
5. In a set of soft sugars from two refineries in which samples had been dried to lower moisture levels, the titratable acidity was significantly less in the lower moisture samples; but the rate of color increase remained high. This again shows that moisture is not the only determinant of color increase on storage, but that it may be a better predictor of acidity increase on storage. A refinery that tended to produce sugars that develop high acidity on storage could ameliorate the problem by producing a lower moisture sugar. The appearance of the color was different, with the high moisture control appearing redder on storage than the lower moisture sugars from the same batch.

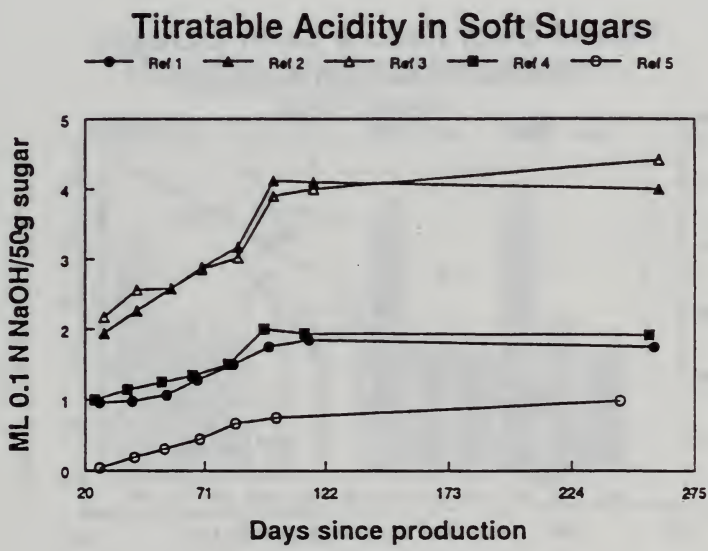
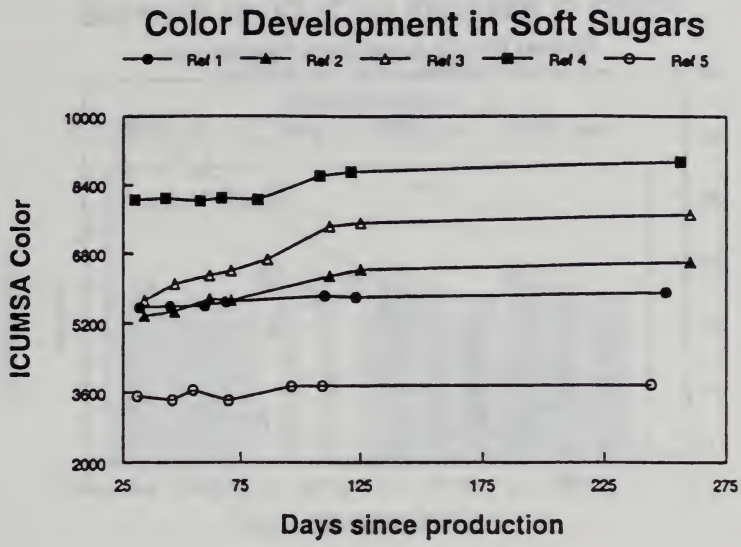
6. Examination of the composition of soft sugars continues, with the goal of identifying acids that contribute to color and/or acid formation. Preliminary data from this report suggest that lactic acid may be a contributing factor. The increase in acids related to sugar degradation was also noted.

Table 1. Equations that define the linear correlation of color development in several soft sugars and the age of the sugar. (X = days)

Ref	Sugar	Start Color	Start Day	End Day	Equation	R ²
A	Med	5552	32	123	$Y=3.14X+5446$	0.965
A	Dk	8075	28	119	$Y=7.87X+7818$	0.974
B	Med	5369	34	125	$Y=11.9X+4945$	0.993
B	Med	4571	28	119	$Y=13.5X+4212$	0.981
B	Med	4632	29	239	$Y=10.5X+4664$	0.927
B	Med	4799	29	239	$Y=8.22X+4865$	0.916
C	Med	5721	34	125	$Y=19.4X+5084$	0.994
C	Med	5248	32	123	$Y=15.8X+4866$	0.965
C	Med	4586	29	239	$Y=9.88X+4586$	0.940
C	Med	3654	29	239	$Y=9.85X+3406$	0.979
D	Dk	8048	30	121	$Y=7.70X+7671$	0.866
E	Lt	3424	18	109	$Y=2.87X+3455$	0.954
F	Lt	1477	112	275	$Y=4.70X+946$	0.962
F	Lt	1582	114	267	$Y=5.18X+995$	0.956
F	Lt	894	84	246	$Y=3.36X+595$	0.982
F	Lt	2149	112	275	$Y=5.28X+1536$	0.973
F	Lt	2124	114	267	$Y=5.76X+1488$	0.979
F	Lt	1958	106	253	$Y=5.17X+1438$	0.980
F	Lt	1943	84	246	$Y=4.66X+1540$	0.997
G	Med	3891	1	110	$Y=6.21X+4004$	0.907

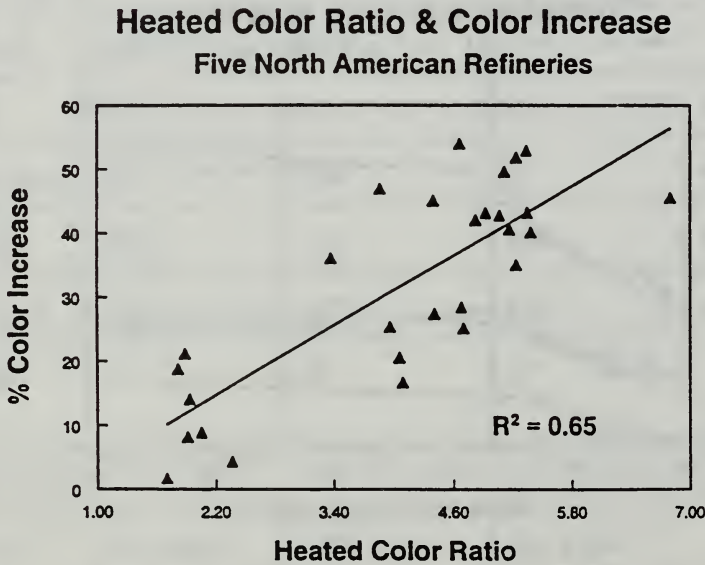
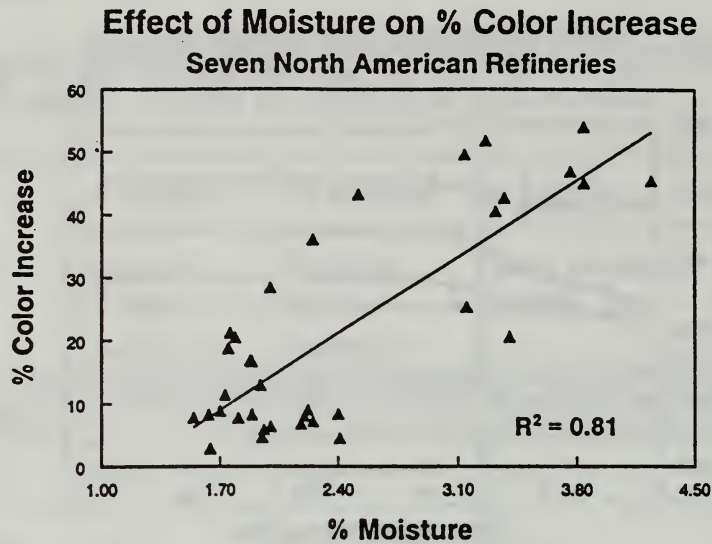
Table 2. Organic and phenolic acids in a soft sugar at two moisture levels at 4 and 14 months of storage. Results are reported as ppm on sugar.

Compound	4 Months	14 Months	4 Months	14 Months
	Control, 3.14% moisture	Control, 3.14% moisture	Low moisture, 1.76%	Low moisture, 1.76%
Organic Acids				
Lactic acid	266	524	271	361
Unidentified acid, 5.09 min.	113	309	113	183
Glyceric acid	119	207	116	170
Malic acid	36	41	38	38
Erythronic acid	81	124	93	112
Quinic acid	299	293	304	308
Gluconic acid	133	187	140	188
Xylonic acid lactone	17	49	19	25
Phenolic Acids				
Benzoic acid	not done	0.63	not done	0.97
4-Hydroxy- benzoic acid	not done	1.73	not done	1.02
Vanillic acid	not done	2.90	not done	2.59
Syringic acid	not done	5.61	not done	5.74
4-Hydroxy- cinnamic acid	not done	0.05	not done	0.08



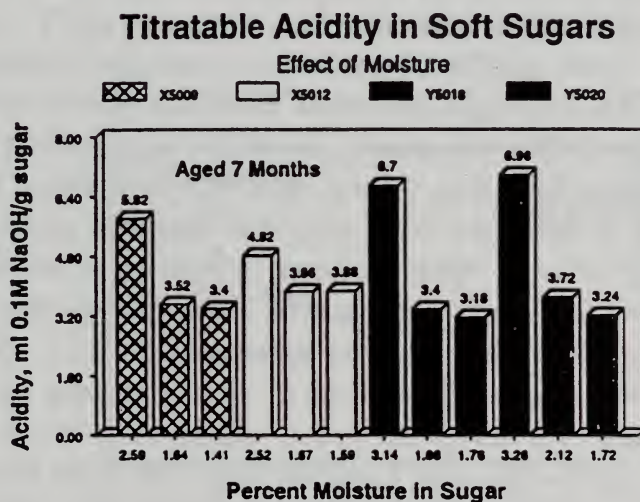
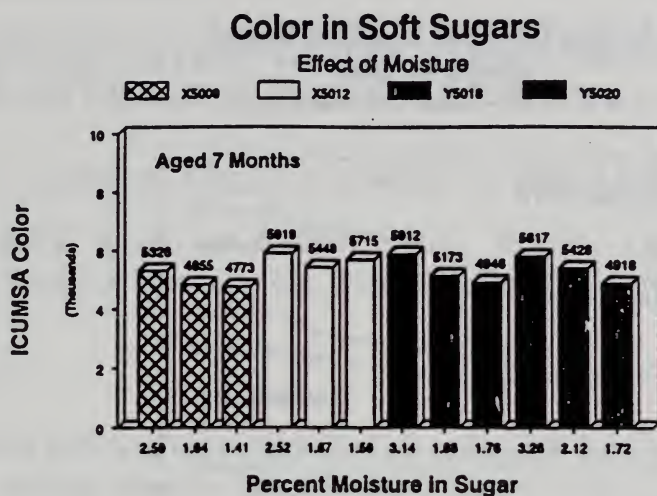
Acidity levels off after about 100 days
Color continues to increase slowly

Figure 1. Changes in color development and titratable acidity in soft sugars.



Color increase correlates well with moisture.
Heated color ratio may be an indicator of color formation on storage.

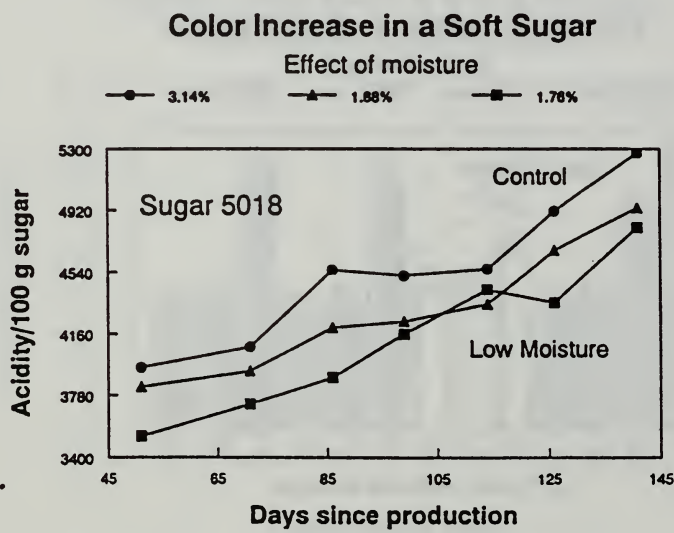
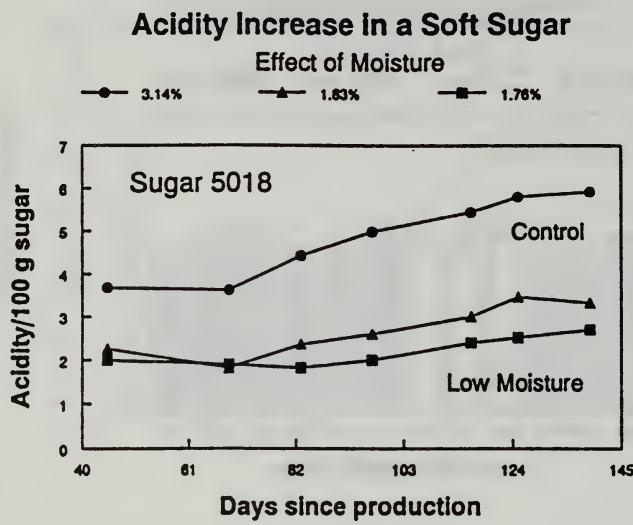
Figure 2. Effect of moisture and heat on color development in soft sugars.



Acidity increases more on storage than color.

Moisture is a major factor in storage changes.

Figure 3. Changes in color and titratable acidity in soft sugars with varying moisture content.



Changes in color and acidity in a soft sugar at three moisture levels.

Figure 4. Changes in acidity and color in a soft sugar at three different moisture contents.

POSTER

A PRELIMINARY STUDY ON THE EFFECT OF SUGARCANE LEAVES AND MUD ON COLOR IN SUGARCANE JUICE

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ABSTRACT

During routine sediment tests conducted on cane juice samples from sugarcane stalks containing varying amounts of leafy cane trash and mud (0, 10, 20, and 30%, alone and in combination, by weight of clean cane stalks of the variety, CP 70-321), a wide range in color was noted in the supernatant which did not correlate to the sediment load in the juice. From these observations, a series of experiments was conducted to quantify the effect of leafy cane trash and mud on juice color. Results confirmed the deleterious effect of leafy cane trash, to include sugarcane leaf blades and sheaths but no tops, both desiccated and fresh, on juice color, with an approximate 6-fold increase in color over the range in leafy cane trash studied. The effect of leafy cane trash on color appeared nonlinear: color increased at a greater rate with each incremental increase in the level of cane leafy trash. On the other hand, mud (Mhoon silty clay loam with approximately 33% moisture) alone showed a decolorizing effect, due, undoubtedly, to the ion exchange properties of the soil type. Further, the effect of mud on color appeared linear: a decrease of 1.6% in color for each 1% increase in mud added to the cane sample. Leafy cane trash and mud in combination (equal amounts of both leafy cane trash and mud by weight of cane up to a total of 30% trash) showed the opposing effects of the two components: color increased with an increase in total trash but not as much as with the leafy cane trash alone. The overall effect was nonlinear. In summary, it appears that in these preliminary studies leafy cane trash added significant colorant to cane juice while heavy textured soil, i.e., silty clay loam, helped to decolorize cane juice.

INTRODUCTION

Field trash is defined as leaves, tops, dead stalks, roots, soil, etc. delivered together with cane (3). Legendre (6) noted that field trash has increased as a result of

mechanical harvesting. Prior to 1943 in Louisiana, the average trash in hand-cut, hand-stripped, and hand-loaded cane was less than 1.9% (6). However, since 1943, field trash levels have increased to over 10% in cane cut and loaded by mechanical means. The same trend has also occurred in Florida.

In studies in South Africa (2), it was reported that with each 1% addition of tops to clean cane the color of clear juice was increased by 1.3% while with each 1% addition of mud to clean cane the color of clear juice was increased by 3.6%. Subsequent studies by Purchase, et al. (9) in South Africa showed that trash contributed substantial color and turbidity to juice. He found that trash appeared to contribute more color than tops alone but when results were normalized there was a linear relationship between color in juice and extraneous material. The composition of the trash was not specifically defined in this work, but based on the nature of the experiments, it probably did not contain field soil or mud. In Australia, Ivin and Doyle (5) defined trash as incorporating the remains of leaves attached to the cane stalk but not including field soil. Further, tops were defined as that portion of the cane stalk above the break point approximately 10 inches in length, minus the top leaves normally cut and blown clear by the cut-chop (combine) harvester. They found that juice color increased an average 25% with the addition of 6% trash (i.e., a 4.2% color increase for every 1% trash). They also noted a 12% increase in juice color by the addition of 6% green tops (i.e., a 2% increase for every 1% tops); however, the increases in juice color were highly dependent on the variety of cane as well, with tops contributing from as little as 2% color in one variety and as high as 29% color in another. The range for trash effects alone on color by variety was from a low of 9.8% to as high as 47.6%

Studies designed to measure relative changes in juice quality are conducted on a continuing basis at the Sugarcane Research Unit, Houma, Louisiana on first-ratoon cane. During routine sediment tests on cane juice, a wide range in cane juice color was noted; color did not always correlate positively to the sediment load in the juice. A series of experiments were set up to quantify the effect on cane juice color of various concentrations of leafy cane trash alone, mud alone, and a combination of leafy cane trash and mud.

MATERIALS AND METHODS

In the present study, leafy cane trash was defined as containing approximately 60% desiccated and 40% green leaves. The type of field soil (mud) added was Mhoon silty

clay loam (Fine-silty, mixed, nonacid, thermic, Typic, Fluvaquents) with approximately 33% moisture. To clean sugarcane stalks of the variety, CP 70-321, varying amounts of leafy cane trash and mud (0, 10, 20, and 30%, alone and in combination, by weight of cane) were added. Approximately 100 hand-stripped stalks of sugarcane were shredded through a pre-breaker. To weighed quantities of the clean, chopped cane, the stated proportion of leafy cane trash or mud or the combination of leafy cane trash and mud was added and the mixture homogenized by passing it once again through the pre-breaker. A 2.2 lb subsample was pressed for 2 minutes, 15 seconds, in a 3-basket hydraulic press at 2,500 lb/in². Each treatment was replicated six times. Fifteen ml of expressed juice from each subsample was centrifuged for 10 minutes at 3000 RPM according to the sediment test described by Birkett (1).

The supernatant was analyzed for color at 420 nm, which is the standard wavelength for ICUMSA color determination (3), and at 560 nm. Samples were filtered on 0.45 μ membrane for the determination. A high color reading at 560 nm relative to 420 nm is said to be a measure of the relative amount of very high molecular weight (MW) colorant (7). There was insufficient juice sample to adjust pH to 7.0, so all color measurements were taken at "natural pH". It is possible that leafy trash and/or mud can change the pH of juice samples, resulting in lower colors at lower pH and in higher colors with higher pH.

RESULTS AND DISCUSSION

Figure 1 shows, in bar graph form, the effect of leafy cane trash, mud, and the combination leafy cane trash/mud mixture on the color of cane juice. Figure 2 shows the effect on color when the data are normalized by the treatments. The mean data for all treatments are shown in Table 1. The deleterious effect of leafy cane trash on juice color was confirmed, with more than a 6-fold increase in color over the treatment range studied. There was a 13% increase in juice color for every 1% leafy cane trash added, up to the 10% level, which is within the range of the Australian experience (5). Mud alone showed a decolorizing effect, due, undoubtedly, to the ion exchange properties of the soil type. The effect of mud was linear with an approximately 1.6% decrease in color per 1% of added mud up to the 30% level. This was contrary to the results found in South Africa where mud also increased the color of juice (2). The results for the combination effects of leafy cane trash and mud mixture reflect the opposing effects of the two components. Color increased from the control but not as much as with leafy cane trash alone because of the decolorizing

effect of the mud. The effect, however, was not linear with the rate of color increase going down as the proportion of total trash increased. There was an 11.5% increase in juice color for every 1% combine trash added, up to the 10% level.

Further, in comparison with untreated juice, there was a trend toward more high MW colorant in juice with leafy trash and less in juice with mud (data not shown). The mud component also appeared to remove high MW colorant in the leafy trash/mud combination. In this preliminary study, it appears that leafy trash not only increases overall color but also increases the load of high MW colorant.

These results show that the components of trash can have different effects on cane juice color, and it is important to define the composition of the trash. Other contributing factors may include the cane variety and type of soil. In this study, only one cane variety was examined, CP 70-321, a variety with low juice color and low phenolics, known color precursors (4, 8). Further, the Mhoon soil series, with its high level of clay, has ion exchange properties enabling it to remove color from the juice. Therefore, the soil type is obviously another important factor in determining the effect of trash under field conditions.

However, the harmful effects of mud, which include contributing to sugar losses in bagasse and filter press mud, increased turbidity and ash, wear and tear to equipment, lowered fuel value of bagasse, etc., would still dictate care in delivering clean, fresh cane to the factory for processing.

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Table 1. Average values for color of centrifuged cane juice determined at 420 nm in the presence of various admixtures of leafy trash and mud (Variety CP 70-321).

Percentage added	Mud	Leaves	Mud + Leaves
0	2,910	2,910	2,910
5 + 5			6,208
10	2,554	6,665	
10 + 10			7,084
20	1,799	12,148	
15 + 15			7,645
30	1,315	19,432	

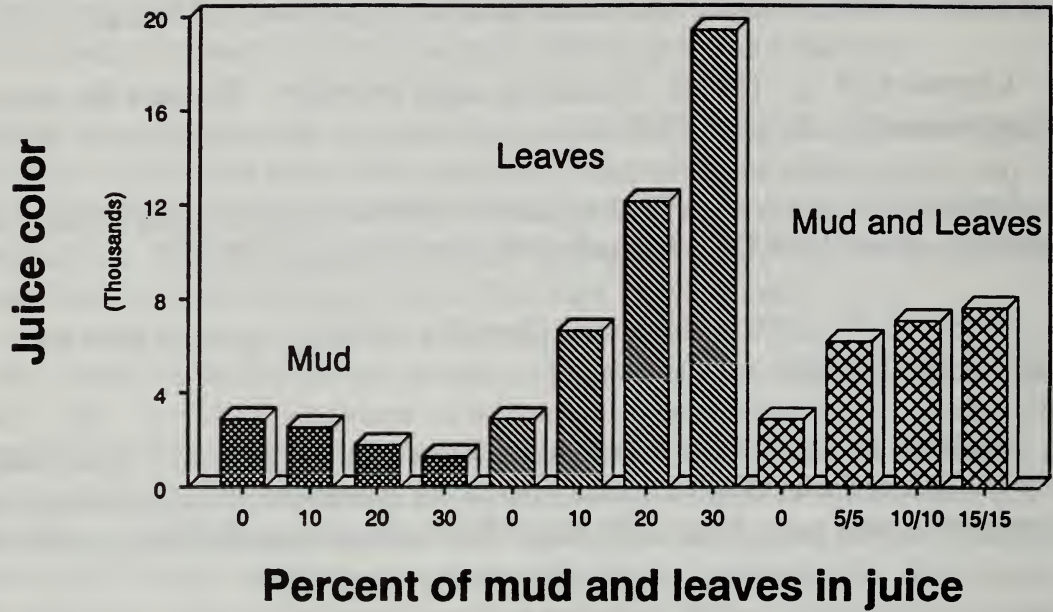


Figure 1. Cane juice color. Effect of extraneous material.

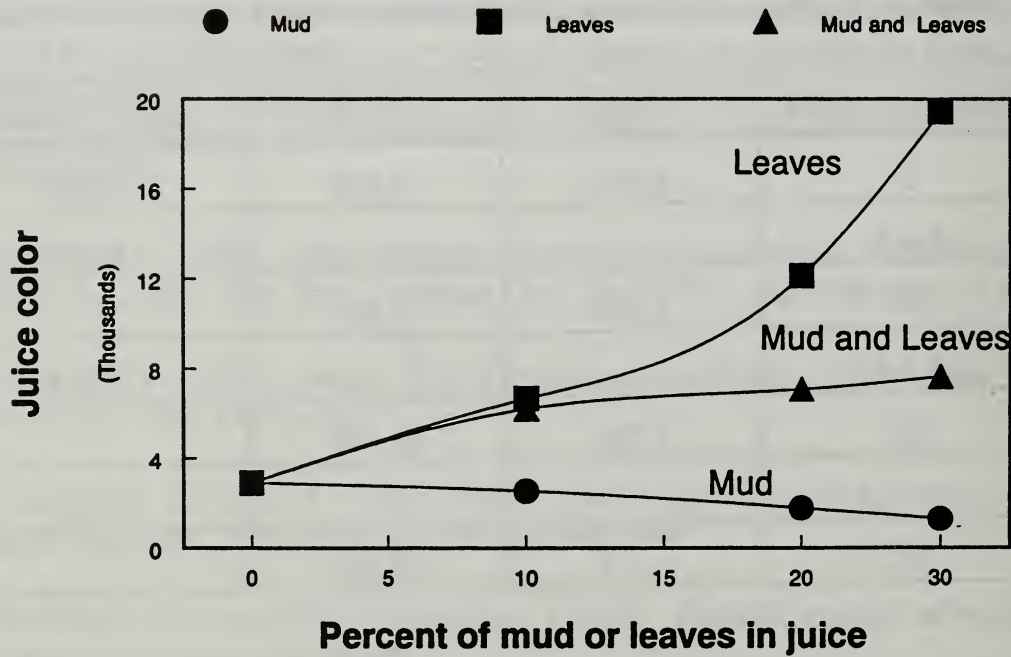


Figure 2. Cane juice color. Effect of trash.

POSTER

ANION COMPOSITION OF RAW CANE SUGARS

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ABSTRACT

A large set of raw sugar samples, from a variety of origins and of varying quality, were analyzed by anion-exchange chromatography with electrometric detection, for chloride, acetate, sulfate, phosphate and other organic anions. Experimental details for this analysis are reported. Sources of these various anions are outlined. Differences in composition are emphasized and explained. The several effects of sulfate ion are emphasized.

INTRODUCTION

A "library" of raw sugars is maintained at S.P.R.I., Inc., containing sugars of various origins, of a wide range of quality, manufactured under a variety of conditions. As raw sugar samples are received, they are analyzed for color, pH, HPLC sucrose, glucose and fructose, polysaccharides, starch, dextran moisture and other tests as appropriate, and stored in the raw sugar library. (A white sugar library is also maintained). It was of interest to determine the anion composition of a range of raw sugars, particularly the sulfate concentration; the library provided an appropriately wide range of sugars. Some are high pol raws, some high quality; others are very dark, low pH sugars; some have been stored correctly, others stored at high temperature and humidity before being sent to S.P.R.I. Some are from factories that make raw sugar only; others are from factories that have a refinery attached (white-end refinery) and others from factories that make a combination of plantation white and raw sugars.

METHODOLOGY

Anion exchange chromatography

Dionex liquid chromatograph

Column: Ion PaC AS-11

Mobile phase: Water/NaOH gradient

Detector: Conductivity with solvent ion suppression

Integrator: Dionex ASI

IONS OF INTEREST

Chloride is the major anion in sugarcane juice, and in raw sugars except for those made with a sulfitation process.

- Comes from cane juice
- Levels in raw sugar range up to 0.1% for very high ash raw
- Used as a marker in refinery process

Phosphate is important in process, for good clarification and color of raw sugar. Levels in raw sugar range up to 50 ppm P, generally below 30 ppm P.

- Irrigation tends to increase all ash component levels
- Important for water run-off
- Source is soil (fertilizer) and cane plant; and added phosphate, if used in clarification process

Acetate is generally a product of bacterial contamination, or, in factories with diffusion, of deacetylation of bagasse.

- Can lower pH if present in high concentrations. Concentration is generally below 200 ppm, but varies widely.
- Can increase corrosion, at high levels
- Important to provide background for good flavor

Sulfate is the major anion in sugars (white) made by sulfitation process, i.e., addition of SO_2 , bisulfite or hydrosulfite. SO_2 reacts with color molecules to bleach them and convert them (reduction) to colorless form, in a redox process whereby sulfite is oxidized to sulfate. The reduced color molecules can undergo reverse reaction to form color again, in presence of air (oxygen) and heat. This is one reason that sulfite process should not be used in raw sugar manufacture: the color returns again in the refinery, and the high residual sulfate remains.

Combined production of plantation white and raw sugar: high sulfate levels are found in raw sugar because white run-offs are recycled to raw sugar production.

White end refinery: if plantation white is remelted, color may reappear, and high sulfate concentration will be recycled back to raw sugar manufacture. If sulfite salts

are used in pans, to bleach or to lower viscosity: same effect - color in raw sugar appears low, but then darkens (color reappears) in the refinery.

Problems with high sulfate levels in raws:

- increased scaling
- ion exchange resins blocked irreversibly - fouling
- bone char decolorization sites blocked
- calcium sulfate precipitate can cocrystallize in raw sugar, and create turbidity

RESULTS: Average values for sulfate, ppm on sugar

Factory type	Location	Soil	Sulfate, ppm
Raw sugar only	Louisiana	med-low sulfate	200
	Florida	high sulfate	600
	Australia	med-high sulfate	400
	Central America	med-low sulfate	200
	Central America	med-high sulfate	500
	Caribbean	high, if volcanic	400-800
Raw sugar and plantation white	Central America	low-med sulfate high, if volcanic	600-1200
	Argentina	low-med sulfate	800
	Peru	high sulfate	1500
	Mauritius	med-high sulfate	850-950
	Caribbean	High, if volcanic	1100-1200
	Thailand	variable	800-1000

CONCLUSIONS

- The major ash component, chloride, varies with source of cane and total ash level in juice. Will increase in cane from irrigated areas.

- Sulfate levels in raw sugars are normally below 500 ppm, except for high ash soil areas, volcanic soils and intensely irrigated land.
- Sulfate levels in raw sugars increase sharply (>800) if sulfite is used in process (often called “viscosity lowering additive”) or if plantation white, sulfited, streams are recycled back to the factory.
- Acetate levels are generally low, <200 ppm, except in raws made with cane diffusion, or with high levels of bacterial infection.

POSTER

IMPROVING THE QUALITY OF DATA WITHIN A FACTORY LABORATORY USING "LIVE" SAMPLES

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ABSTRACT

Routine samples of beet sugar molasses were double-sampled each shift and one sample reserved for "re-check" analyses at a later date. When a sufficient pool of re-check samples had been established, coded re-check samples were randomly submitted each shift and analyzed as if they were routine samples. The code was broken and the data compared with the original analyses to identify areas for improving the sampling and analytical processes. Data are presented for RDS (refractometric dry substance) determination on molasses for two locations using two different types of refractometers. Typical SPC methods were used to chart progress; subsequent analyses of the data indicated opportunities for improvement.

POSTER

POLYMERS IN SUGARBEET PROCESSING

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ABSTRACT

The nature and concentration of polymer compounds present in the various steps of sugarbeet processing, i.e. from raw juice up to white sugar and molasses, have been determined.

Additionally, the composition of the polysaccharide fraction among these polymers will be presented.

For purpose of isolation of polymers, techniques like alcohol precipitation and dialysis have been applied. HPAEC-PAD has been used for the analysis of the saccharide composition of the polysaccharides.

The resemblance of polysaccharides isolated from white sugar to those isolated from acid beverage floc points out that hairy regions of pectin may take part in the formation of floc particles.

INTRODUCTION

Polysaccharides extracted from beet tissue during the diffusion process are important in further processing for several reasons (1):

- They add to non-sucrose solids content and decrease purity;
- They can decrease filtration rates as a result of increased viscosity;
- Polysaccharides can have an effect on polarization;
- They may influence product quality with respect to both molasses (fermentability, nutritive value) and white sugar (formation of beverage floc in soft drinks and in alcohol-containing drinks).

Vogel and Schiweck (2) isolated and characterized the polysaccharides in juices and syrups from sugarbeet. Their results showed that the pectin part in raw juice is degraded (or removed!) almost completely during the juice purification. The remaining polysaccharides were recognized as hemicellulose, which apparently possessed a high stability against heat and alkali.

This paper will discuss our findings on the course of polymers in general and polysaccharides in particular throughout the sugar manufacturing process.

The quantitative amounts of polymers present at the different processing steps have been determined. Also the amount and composition of the polysaccharide fraction are reported.

The characteristics of polysaccharides present in process streams and observed in white sugar are compared to those of polysaccharides isolated from acid beverage floc.

It must be noted that the samples which are investigated in this study, do not necessarily correspond to each other. For example, the molasses sample was taken at the same time as the raw juice sample, whereas in the process this molasses will have been related to raw juice produced more than two days earlier.

EXPERIMENTAL

For the isolation and characterization of polymers generally available techniques and methods have been used. Figure 1 outlines the sample preparation and analysis methods applied for liquid samples. Polymers from white sugar were obtained by dialysis using semi-permeable membranes with cut off at molecular weight 12-14.000 Dalton.

RESULTS AND DISCUSSION

Course of polymers throughout the process

Figure 2 depicts the amount of polymers present at different stages in the process as well as their global composition.

These data clearly demonstrate that in juice purification the major part of polymers are removed. Besides the substantial decrease of uronic acids, particularly the removal of protein explains the drastic decrease of the polymer content.

It is interesting to note that after juice purification the content of polymers gradually increases. This may be explained by formation of polymeric Maillard reaction products, due to prolonged heating of the juices/syrups during further processing.

The polysaccharides remaining after juice purification appeared to be rather stable under the applied process conditions.

Characterization of polysaccharides

The sugar composition of the polysaccharides have been determined; Table 1 summarizes the results.

The removal of pectin substances in juice purification is confirmed, although the polysaccharides in subsequent processing steps still contain some uronic acids.

The major saccharides found after TFA hydrolysis are L-arabinose and D-galactose. Polysaccharides which survived juice purification do not undergo any further change, demonstrating their heat stability.

The polysaccharide compositions we have determined are in close agreement with those found by Vogel and Schiweck (2), except for the uronic acids content. It seems that they have underestimated the uronic acids content of the polysaccharides, maybe as a result of the different isolation method for polysaccharides they have applied (ultrafiltration and subsequent dialysis).

Polysaccharides in white sugar and floc

Polymers in crystalline sugar were obtained by dialysis and typically amounted to 25-30 mg per kg sugar (3 different samples). The average molecular weight of the polymers was approximately 120 kDalton, as determined by HPGPC (on Bio-Gel TSK columns).

From the sugar analysis after TFA hydrolysis of the polymers it can be concluded that polysaccharides explain 42 % of the polymers in white sugar.

Thus, 58 % of the polymers consist of unidentified non-sugar polymers, most likely melanoidins (Maillard reaction products).

During the sugarbeet campaign weekly composite samples of white sugar are analyzed for the potential to form acid beverage floc. For that purpose the ICUMSA floc test, Coca-Cola method, is used. Usually, after the tenth day's observation, the floc is ranked as negative or turbid. According to the rating system for floc the number = 0, which expresses the good quality of the sugar. The jars of the floc tests were set aside and after several months some floc appeared. This floc formation after prolonged testing was certainly not of microbial origin.

After the campaign precipitated floc particles from various jars were collected. Floc polysaccharides were precipitated with alcohol and, after TFA hydrolysis, an indication of the saccharide composition was obtained by HPAEC.

In Table 2 this indicative composition of polysaccharides in floc is compared to those determined in white sugar respectively standard liquor. Although the samples were not taken in chronological order, the resemblance between the polysaccharides in process, sugar and floc is striking.

The saccharide composition of the isolated beetsugar polysaccharide quite well agrees with that of the so-called hairy regions in pectin (3).

CONCLUSIONS

In beetsugar manufacture the major part of extracted polymers, i.e. protein and pectin, is removed in juice purification. The composition of the remaining polysaccharides look like that of the hairy regions of pectins. These polysaccharides appeared to be stable under (further) process conditions and finally end up in molasses (with a negligible amount in white sugar).

White sugar contains approximately 25-30 mg polymers per kg sugar, from which 42 % can be characterized as polysaccharides resembling the hairy regions of beet pectin. In evaporation and in the vacuum pan station additional polymeric compounds are produced, most likely due to Maillard reactions of reducing sugars and amino acids.

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Table 1. Sugar composition of polysaccharides.

Saccharide, mole %	raw juice	thin juice	thick juice	std. liquor	molasses
L-Rhamnose	1	2	2	2	1
L-Arabinose	18	39	37	40	34
D-Galactose	13	26	24	27	20
D-Glucose	7	9	10	8	12
D-Mannose	2	6	6	6	8
D-Xylose	2	2	2	4	1
D-Fructose	1	0	0	0	0
Miscellaneous	2	2	2	2	3
Uronic acids	54	14	17	11	21

Table 2. Comparison of polysaccharide composition in process, sugar and floc.

Saccharide, mole %	std. liquor	white sugar*	floc
L-Rhamnose	2	4	4
L-Arabinose	40	36	54
D-Galactose	27	23	8
D-Glucose	8	18	8
D-Mannose/D-xylose	10	6	11
D-Fructose	0	0	0
Miscellaneous	2	3	5
Uronic acids	11	10	10

* average of three different sugar samples

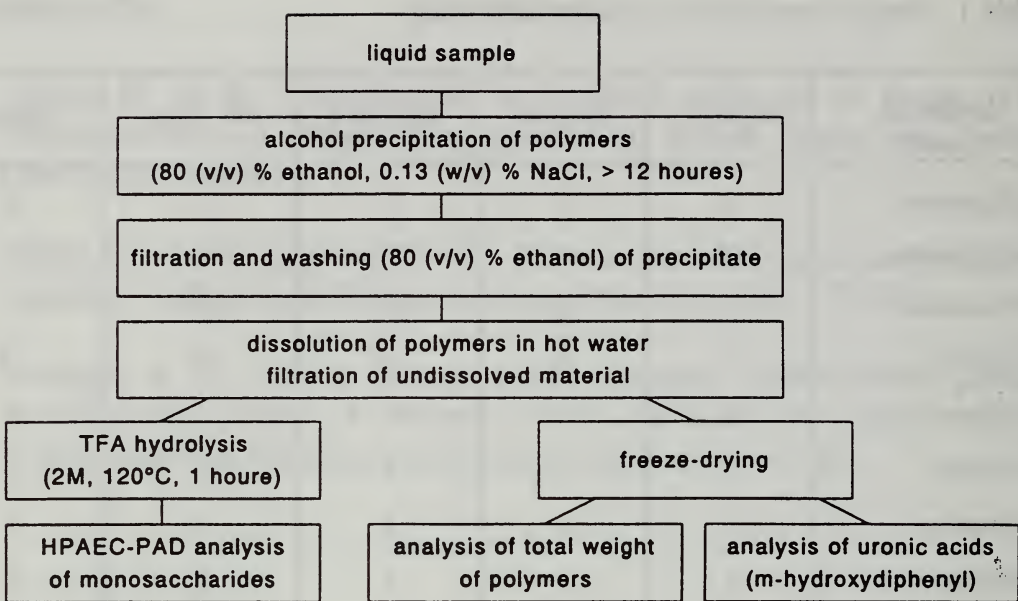


Figure 1. Method of isolation and analysis of polymers.

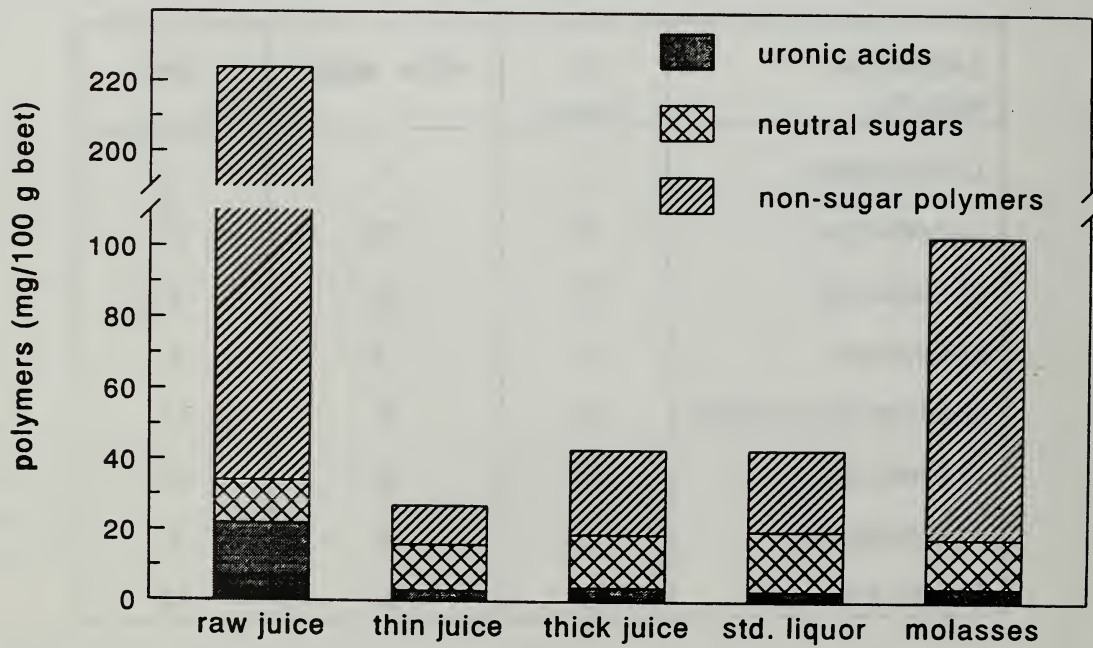


Figure 2. Global polymer composition in beetsugar manufacture.

POSTER

THE USE OF DIFFERENTIAL SCANNING CALORIMETRY AND THERMO-GRAVIMETRIC ANALYSIS TO CHARACTERIZE THE THERMAL DEGRADATION OF CRYSTALLINE SUCROSE AND DRIED SUCROSE-SALT RESIDUES

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ABSTRACT

Impurities such as salts are known to increase the thermal degradation of concentrated sucrose solutions in the sugar factory and refinery. This leads to chemical losses of sugar, formation of degradation products and reduction of unit process efficiencies. Data is required to better predict the kinetics of sugar losses, and also physico-chemical and thermal data for engineers and designers to make improvements in thermal process operations such as clarification and evaporation in the sugar industry. Thermal degradation of pure sucrose solutions (100 °C; ~65Brix) in the presence of KCl, LiCl, NaI and Na₂B₄O₇ salts was investigated. All salts increased sucrose degradation as monitored by polarimetry. Colored degradation products were only formed in the presence of Na₂B₄O₇. Thermal degradation characteristics of crystalline sucrose and dried, crystalline residues from sucrose-salt model solutions heated at 100 °C for 0 and 3h, were further investigated by DSC, TG and DTG analyses. Rate of heating was 15 °C/min from 50 to 500 °C. DSC and TG studies confirmed the catalytic nature of salts on the thermal degradation of sucrose. Salts affected thermal degradation in various ways. The initial condensation degradation reactions were more rapid than expected and subsequent elimination reactions were slower. Thermal analysis results indicate that complexation between the salt and sucrose is occurring.

INTRODUCTION

Recent studies by Eggleston et al., (1,2) described the catalytic effects of various salts on the thermal degradation of concentrated aqueous solutions (~65Brix) of sucrose, and initial degradation kinetic rates as monitored by pol and ion chromatography were reported. However, the physico-chemical behavior of sucrose under thermal

conditions would be better defined using thermal analysis, such as DSC and TGA, which are particularly useful for indicating the temperature range and the rate of thermal processes. Relatively little has been published on the thermal properties of sucrose, industrial raw and brown sugars, syrups and honeys, and for the effects of salts and other process or food components even less (3,4,5). This study uses model sucrose-salt systems to determine the effects of salts on the initial degradation of ~65Brix sucrose solutions at 100 °C. Salts studied included LiCl and KCl, water structure enhancer and breaker, respectively (2), and $\text{Na}_2\text{B}_4\text{O}_7$ and NaI, salts known to complex with sucrose. In order to more fully define the effect of salts on the thermal behavior of sucrose, select model solutions were carefully dried and investigated with DSC, TG and DTG techniques.

EXPERIMENTAL

Sucrose Degradation Model Systems consisted of molar sucrose-water-salt/solute ratios of 1:10:0.05M respectively (equivalent to a sucrose concentration of ~65Brix). Portions (3 mL) of the reaction solutions were distributed in 5 mL Pierce Reactivials™, and sealed with Teflon septa. Reactivials were then flushed with nitrogen and placed in an oven at 100 °C for the required time, cooled and stored at -43 °C until analysed. See (1) for full method.

Polarimetry. Results are expressed as specific rotations. See (1) for method.

Sample Preparation for Thermal Analysis. Model sucrose-salt solutions at 0 and 3 h reaction time, and crystalline salts, were placed in a vacuum oven at 50 °C until dried to constant weight.

Thermal Analysis were performed with a TA Instruments 2100 Thermal Analyzer. DSC analyses were performed using a module equipped with a dual-sample cell and with auto-sampling capability. Aluminum hermetically sealed pans to minimize detrimental effects of volatiles on the cell were used. The heating program was from 50 to 500 °C at 15 °C/min. Nitrogen (100 mL/min) was the purge gas. Sucrose samples were weighed accurately and, if necessary, were later normalized to 5.0 mg for comparability. TG analyses were made with the Automated Hi-Res™ TGA 2950 system. Samples were heated at 15 °C/min. Sucrose samples for TGA weighed from 10-12 mg.

RESULTS AND DISCUSSION

Thermal Analysis of Crystalline Sucrose. The sequence of thermal degradation of crystalline sucrose was determined by DSC, TG and DTG analyses as shown in Fig 1. The concurrent and consecutive individual reactions which take place within each thermal event are characterized by changes in mass (TG) and/or energy (DTG and DSC). The temperature of individual thermal events is dependent on the rate of heating. Additional thermal runs were undertaken to assess the physical appearances of the sucrose at significant thermal steps; results are indicated on Fig 1, and confirmed the individual thermal processes. Richards and Shafizadeh (3) observed that the first TGA weight loss peak represented condensation reactions leading to oligomeric and polymeric products, and they assumed the second weight peak loss represented "complex elimination reactions which yield solid char plus a wide range of volatile products".

Concentrated sucrose-salt solution model systems: thermal degradation characteristics. Very little sucrose degradation, as monitored by pol, was observed in the model system (65Brix) containing only sucrose (control) over the first 4 h, with subsequent accelerated degradation from 4 to 6 h. Both KCl and LiCl increased degradation, with no color formation over 6 h reaction time. NaI did not markedly affect the initial degradation over the first 2 h; no color was formed. Although $\text{Na}_2\text{B}_4\text{O}_7$ did not appear to significantly affect degradation, a very pale color formed after only 1 h and the reaction solution was a very dark golden-brown color after 6 h, which signified that marked degradation had occurred. Pol is a very precise technique to measure sucrose degradation, provided that no other optically active compounds are present. However, if traces of degradation compounds with a high positive pol are present, the pol kinetic change will be suppressed, and this is the likely explanation (see 1,2 for further information).

Differential scanning calorimetry. The normalized DSC thermograms of the model sucrose-salt samples at 0 h indicated that the majority of the sucrose was in the crystalline state. However, slightly reduced melting endotherm peak temperatures, from the crystalline melting endotherm of the control, may have reflected small amounts of amorphous sucrose, although a more likely explanation is that the salts thermally destabilized the sucrose to lower temperatures as significantly less enthalpy was required. Furthermore, melting endotherm peak temperatures of the 3 h dried samples were consistently shifted to even lower temperatures than the 0 h samples, which further suggested that thermal degradation of sucrose was facilitated in the

presence of these salts. This facilitation could be attributable to the presence of catalytic degradation products formed over the 3 h reaction time of the model sucrose-salt solution (before drying) or to direct complexation of the salt and sucrose in the crystalline state.

Thermogravimetric analysis. DTG peak temperatures for the 0 h samples were all markedly shifted to lower temperatures than the control and those for the 3 h samples to a further extent, which confirms the DSC evidence that salts and sucrose thermal degradation products, respectively, destabilize sucrose and facilitate its thermal degradation. Haroun et al., (4) observed that brown factory sugars with relatively high ash contents (~1.3-2.0%) had DTG endotherm peak temperatures shifted to lower temperatures than the pure sucrose peak. The rate of weight loss values for the maximum peaks (R_{max}) of all the sucrose-salt residues were found to be higher than for the control. Rate is an indicator of how easily the thermal process is occurring and the higher values further reflected the facilitation of the sucrose thermal processes by the presence of the salts. From 0 to 3 h R_{max} decreased for all samples and this effect was strongest in the sucrose-salt residues. The degradation products already present in the samples most likely created a more viscous "reaction syrup" which caused a slower rate. In general, the minimum peak rates did not vary significantly from 0 to 3 h, which suggested that impurities from sucrose degradation have more effect on the condensation reactions prevalent in the first (max) peak.

Comparison of theoretical values. "Expected" or "theoretical" % overall total weight loss from the sucrose-salt (0 h) dried residues were calculated by simple addition of the individual % total weight losses for the crystalline salts and control sucrose. [3h solutions expected losses were not calculated, as the exact amounts of sucrose present were not known due to the formation of small amounts of degradation products in solution.] For all the sucrose-salt residues at 0 h, the observed % total weight loss was always significantly lower than the expected value, which is strongly indicative of the formation of a stronger complex that reduces the overall amount of pyrolysis of sucrose under these crystalline conditions. The expected % total weight loss values for KCl and LiCl at 0h lie within the range of values for the known strong complexors of sucrose in solution NaI and $Na_2B_4O_7$, and the difference between the observed and expected % weight loss values was highest for KCl.

CONCLUSIONS

- * DSC and DTG studies confirmed the catalytic nature of salts on the thermal degradation of sucrose.
- * Salts increased the initial condensation degradation reactions, which created a more viscous "reaction syrup" causing rates/extents of degradation in subsequent elimination reactions to be lower than expected.
- * Observed total % weight losses of 0 h samples were always lower than calculated or theoretical weight losses, and amorphous sucrose effects can be eliminated. This indicates that a complex between the sucrose molecule and salt, which loses less residue on thermal analysis than individual components, has formed in the crystalline state. However, the complex itself represents new material which is capable of thermally destabilizing the sucrose to lower temperatures than for sucrose alone.
- * We believe complexation is occurring, and needs to be further studied at the molecular level, for example, with the use of NMR techniques.
- * The use of DSC and DTG, as reported here, may offer a quick, accurate and routine technique to provide further knowledge of the chemical and physical characteristics of sucrose containing salts or other impurities, which are often encountered in the sugar processing and food industries, and may be of particular interest to industrial processing engineers.

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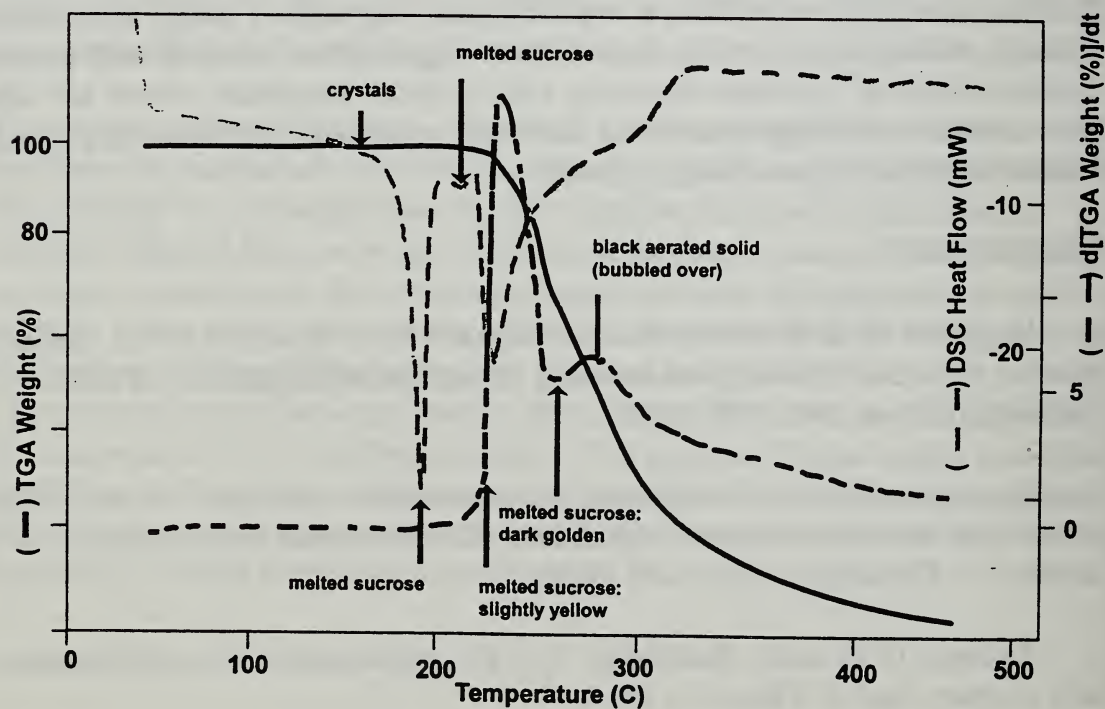


Figure 1. Overlaid TGA, DTG and DSC thermograms of pure, crystalline sucrose.

POSTER

FEASIBILITY OF RAPID YEAST METHOD FOR ON SITE BEET FACTORY JUICE AND CANE REFINERY SYRUP SAMPLE ANALYSIS

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ABSTRACT

Applicability of a rapid yeast quantitation method utilizing hydroscopic membrane technology as a routine on-site tool for analysis of yeast populations in beet factory juice and cane refinery syrups is examined. If feasible, implementation of the method will reduce analysis time from a five to seven day turn around time to a two to three day turn around time. Another benefit of the filtration method is that an increased sample size can be analyzed, thus increasing sample representation.

POSTER

MOISTURE DETERMINATION BY NEW INSTRUMENTAL TECHNIQUES

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ABSTRACT

The official ICUMSA method for moisture determination in sugar products by loss-on-drying requires 3 hours of oven-drying at 105°C followed by an extended cooling period to 2°C above ambient. Available instrumentation is now capable of accurately measuring the small amounts of moisture in white sugar. This presentation reports on the evaluation of two new instruments, which utilize the loss-on-drying method. The Arizona Instruments Computrac MAX-2000 Moisture Analyzer uses a small convection air oven to effect drying. The Instrument Corporation OmniMark I High Performance Moisture Analyzer utilizes four quartz infrared heaters. Drying parameters have been studied in both instruments for white, raw, powdered and soft brown sugars.

The choice of drying parameters plays a critical role in successful moisture determination. Both instruments give results comparable to the oven, with a great savings in time. Precision of measurement using the instrumental methods is at least doubled for white and powdered sugars and approximately the same for raw and soft sugar compared to the oven procedure.

INTRODUCTION

Moisture is an important quality parameter in sugar products, affecting storage stability, packaging, pourability, color formation and microbiological stability. The Commission for Uniform Methods of Sugar Analysis (ICUMSA) officially recommends an loss-on-drying oven method. This method is reliable and repeatable for sugars with a moisture level above about 0.05%, but it is time consuming, requiring a drying time of 3 hours, and a lengthy cooling period. For white sugars, with their lower moisture content, the reproducibility can be poor (Vaccari, et al., 1990, 1993).

In this study, the moisture in white, raw, soft, and powdered sugars was determined by the official ICUMSA oven method (Method GS1/1/3-15) and by two new moisture analyzers.

ANALYZERS

OmniMark Instrument Corp., Denver Instrument Co., Tempe, Arizona, USA

MARK I High Performance Moisture Analyzer

- ▶ 2 year parts and labor warranty
- ▶ Resolution: 0.1 mg
- ▶ Capacity: 100 g
- ▶ Programmability = 10 drying procedures
- ▶ Method: Four quartz infrared heaters and detection of weight loss on drying
- ▶ Temperature range: 30 - 210°C;
- ▶ Temperature sensor: Platinum RTD resistance device
- ▶ Printer: Integral 40-column dot matrix

Arizona Instruments; Phoenix, Arizona, USA

Computrac MAX-2000 Moisture Analyzer

- ▶ 1 year parts and labor warranty
- ▶ Resolution: 0.1 mg
- ▶ Capacity: 40 g
- ▶ Programmability: 31 drying procedures; some basic statistics; graphing functions, security function
- ▶ Method: Loss on drying using a small convection air oven
- ▶ Temperature range: 25 - 275°C;
- ▶ Temperature sensor: Platinum RTD resistance device
- ▶ Printer: Separate Epsom dot matrix printer included in price package

DRYING VARIABLES

Parameters to choose when selecting a method:

Temperature

Time

Sample size

Stand-by temperature

End of analysis mode

End of analysis modes:

- ▶ Time (Not recommended)
- ▶ Constant weight (Slope)
- ▶ Actual slope or rate
- ▶ Calculated slope (Prediction)

Slope/Rate: Percent of initial weight change over a chosen period of time. Test ends when slope conditions met.

Calculated/Prediction Mode: Test ends before actual rate is reached, based on prediction algorithms. Shortens analysis time; may use higher starting temp; avoids sample decomposition.

SOFT SUGAR RESULTS

Comparing the 3 Methods

<u>Sugar</u>	<u>Oven</u>	<u>MAX-2000</u>	<u>Omni-Mark</u>
1	2.41	2.14	1.97
2	3.15	2.98	3.06
3	4.24	3.45	3.80
4	3.37	3.31	3.00
5	3.05	2.95	3.32
6	1.76	1.76	1.89
7	1.64	1.66	1.70
Mean	2.80	2.61	2.68

Parameters

MAX-2000: 10 g; 140°C; Rate 0.005%; standby 103°C; Average time of analysis 11 min

Omni-Mark: 10 g; 140°C/5 min; Slope 0.04%/min; standby 120°C; Average time of analysis 7.5 min.

Results of 10 Paired Comparisons of Soft Sugar Moisture

<u>Sugar</u>	<u>Oven</u>	<u>Omni-Mark</u>	<u>Sugar</u>	<u>Oven</u>	<u>MAX-2000</u>
H	3.92	3.73	A-1	1.94	1.98
J	3.85	3.31	A-2	1.88	1.82
K	3.76	3.39	A-3	1.95	1.92
M	2.25	2.58	A-4	1.63	1.74
R	1.78	1.49	B-1	1.89	1.80
CL	2.18	2.41	B-2	1.96	2.02
CD	1.47	1.58	B-3	2.22	2.50
6	1.89	1.76	B-4	2.40	2.46
7	1.70	1.64	C-1	1.54	1.51
2A	3.15	3.06	D-4	2.00	2.17
Mean	2.60	2.49	Mean	1.94	1.99

There was not significant difference between the oven and the two instrumental methods.

WHITE SUGAR RESULTS

Moisture in a white sugar -- Comparison of methods.

<u>Oven</u>	<u>OmniMark</u>	<u>MAX-2000</u>
0.046	0.050	0.050
0.052	0.049	0.054
0.057	0.040	0.043
0.035	0.045	0.049
	0.045	

Oven	0.048% \pm 0.00947	(C.V. = 19.73%)
OmniMark	0.046% \pm 0.00396	(C.V. = 8.61%)
MAX-2000	0.049% \pm 0.00455	(C.V. = 9.29%)

Effect of Sample Size on Results (Omni-Mark)

<u>Sugar</u>	<u>20g</u>	<u>30g</u>	<u>40g</u>
1	0.035	0.027	0.020
2	0.043	0.024	0.016

Moisture determination of white sugar is sensitive to sample size. Achieving target weight is important, as well as adjusting parameters to give a result comparable to the oven standard.

POWDERED SUGAR RESULTS

Moisture in powdered sugar

<u>Sugar</u>	<u>Oven</u>	<u>OmniMark</u>	<u>MAX-2000</u>
1	0.411	0.366	0.463
2	0.449	0.464	0.599
3	0.482	0.471	0.626
4	0.373	0.354	0.437
5	0.475	0.463	0.372
Over-all	0.438	0.424	0.499

Time OmniMark: 3:24-5:00 min.

Time MAX-2000: 2:55-4:23 min.

Parameters:

OmniMark: 120°C/3 min; slope 0.03%/1.5 min; standby 100°C

MAX-2000: 10 g; 120°C; 95% prediction; 0.01% stability; standby 95°C

RAW SUGAR RESULTS

Comparing the 3 Methods

Results of Paired Comparisons

<u>Sugar</u>	<u>Oven</u>	<u>MAX-2000</u>	<u>OmniMark</u>
Florida	0.193	0.208	0.190
Phill.	0.340	0.383	0.321
Peru	0.182	0.211	0.186
Mexico	0.180	0.190	0.180
Over-all	0.224	0.248	0.219

Parameters:

MAX-2000: 130°C, 0.005% rate; 20 g.

OmniMark: 140°C/4min; slope 0.01%/2min; 20g.

20 Paired comparisons by oven and Omni-Mark showed no significant difference ($P>T=0.5660$). Parameters: 140°C/3 min; slope 0.04%/1.0 min; stand-by 105°C.

Effect of Different Parameters

On Moisture Determination of a Raw Sugar (MAX-2000)

<u>%Moisture</u>	<u>Test Conditions</u>
0.147	100°C; 5.0 min; end test on time; 10 g
⇒0.185	110°C; 5.0 min; end test on time; 10 g
0.191	120°C; 5.0 min; end test on time; 10 g
0.244	130°C; 5.0 min; end test on time; 10 g
0.172	130°C; rate 0.005%; predict mode; 20 g
0.179	130°C; rate 0.005%; predict mode; 20 g
⇒0.184	130°C; rate 0.005%; rate mode; 20 g
⇒0.182	Standard oven conditions

⇒Shows that several parameters can give the “correct” result, but ending on time may cause errors depending on the actual moisture content of the sugar and is not recommended.

Summary of Comparative Data (Over-All Means of Paired Samples)

Sugar	Oven	Max-2000	Omni-Mark
Soft	4.0	2.1	3.9
Powdered	13.7	2.8	6.1
Raw	4.5	5.2	4.4
White	20	10	10

Repeatability (Measure of Precision Within a Laboratory)

Sugar	Oven	Max- 2000	Omni Mark
Soft	2.70	2.46	2.59
Powdered	0.438	0.499	0.424
Raw	0.224	0.248	0.219
White	0.048	0.049	0.046

CONCLUSIONS

- ▶ Precision is doubled for white sugar moisture by instrumental methods.
- ▶ Instrumental methods are more precise and much faster than the oven method.
- ▶ Both instruments give results comparable to the oven method when proper parameters are chosen.
- ▶ Choice of parameters is critical and should always be checked against oven results. Parameters given here should act as guidelines only, since laboratory conditions and type of product can produce slight variations in results.

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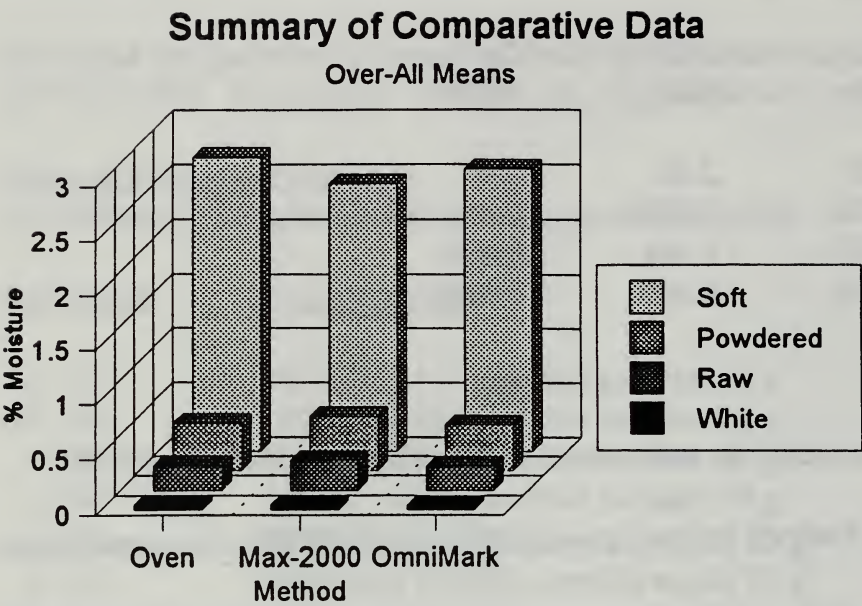


Figure 1. Summary of moisture data as over-all means for four types of sugar products analyzed by three methods.

POSTER

THE BEHAVIOR OF AMINO ACIDS IN CHROMATOGRAPHIC MOLASSES DESUGARIZATION SYSTEMS

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ABSTRACT

Free amino acids in sugarbeet molasses demonstrate a variety of separation characteristics during molasses desugarization by ion exclusion chromatography. Several amino acids are easily removed with raffinate while others behave more like sucrose and elute in the product fraction. Several amino acids appear to accumulate within simulated moving bed desugarization systems. The relationship between amino acid separation characteristics and ionic properties will be discussed.

INTRODUCTION

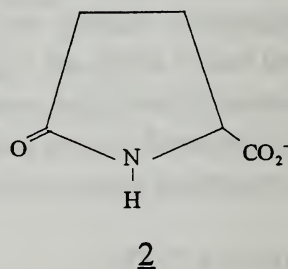
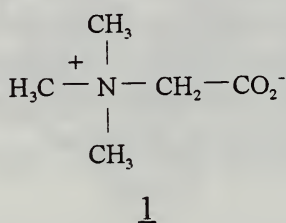
Sugarbeet molasses contains significant quantities of nitrogen compounds including free α -amino acids. Typical levels of α -amino acids and related nitrogen compounds are given in Table 1.

Together the nitrogen compounds listed in Table 1 make up 30-35% of the non-sucrose components of sugarbeet molasses and thus have a significant effect on the desugarization of molasses using simulated moving bed ion exclusion chromatography.

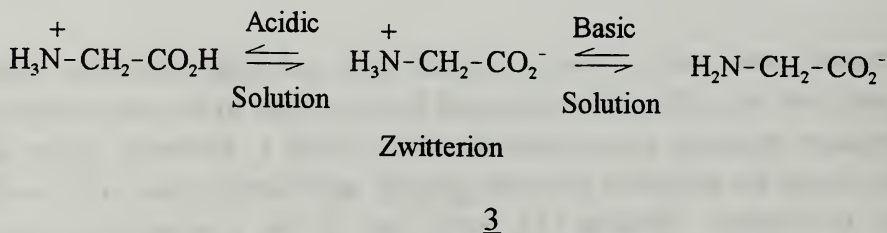
As discussed previously, strongly ionized salts and high molecular weight organic compounds are very efficiently separated from sucrose in the ion exclusion process¹. The common nitrogen compounds listed in Table 1, however, show a variety of behavior during ion exclusion chromatography, undoubtedly due to the wide variation in ionic properties. Betaine (1), being one of the predominant components of sugarbeet molasses, is naturally of great interest and its separation properties have been mentioned previously¹. Betaine at neutral to slightly basic pH values exists as the zwitterion shown in (1). This species is ionic but evidently due to its net zero charge behaves somewhat like a small neutral molecule. Betaine is eliminated with raffinate (salts and large molecules) to the extent of approximately 65% in factory systems, as opposed to elimination values of 95% or higher for common inorganic

salts. The relatively large fraction (35%) of betaine which elutes with sucrose is probably due to the behavior of betaine like a neutral molecule.

PCA (2-pyrrolidone-5-carboxylic acid) is also a significant component of sugarbeet molasses, arising from the loss of ammonia from glutamine during juice purification. PCA, though, has only one functional group (carboxylic acid) that is easily ionized under chromatographic conditions and exists in neutral solution as the carboxylate anion (2). This species behaves like any other anionic substance and elutes with salts in the raffinate (at 95-98% elimination).



Unlike betaine which is locked in a zwitterionic structure by the quarternary ammonium salt functional group, and PCA, which behaves like a simple carboxylic acid due to the lack of a basic amine function group, the α -amino acids and γ -aminobutyric acid possess both basic and acidic functional groups and can exist as an anion, cation, or doubly charged zwitterion depending on solution pH. This is illustrated for the simplest α -amino acid, glycine, which can exist as the zwitterion 3. The solution pH at which a particular amino acid exists predominantly as the zwitterion,



with a net zero charge, is referred to as the *isoelectric point* of that amino acid. Because the 15 amino acids commonly found at significant levels in sugarbeet molasses possess a variety of structural differences and differing isoelectric points, they demonstrate a full range of separation characteristics in ion exclusion process chromatography. The observed behavior in process-scale systems, which can range

from very efficient elimination with raffinate to poor elimination and elution with sucrose, will be the topic of the remainder of this discussion.

DISCUSSION

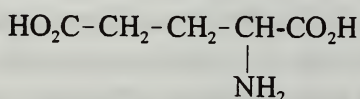
Amino Acid Elimination in Ion Exclusion Chromatography

Typical levels of amino acids in sugarbeet molasses are given in Table 2 which shows values in feed molasses and sucrose eluent stream (extract) for a factory simulated moving bed separation system over an eight hour period. Levels are given in both percent based on refractometric dissolved solids and percent based on non-sucroses. Notice that two amino acids, glutamic acid and aspartic acid, are present at much lower levels (based on solids) in extract than feed while others such as serine, tyrosine, valine, and isoleucine are actually present at significantly higher levels in extract. Amino acid levels calculated based on non-sucroses show these differences even more dramatically. Glutamic and aspartic acid levels based on non-sugars, show some decrease across the separator indicating that they are removed somewhat more efficiently than total non-sucroses (which are removed at levels of about 87% in this example). Most other amino acids, however, are actually present at higher levels, based on non-sucroses, in extract than in feed molasses. Higher levels of amino acids arise from these substances being more poorly eliminated than other non-sucroses resulting in enrichment of the extract stream non-sucroses in amino acids. Notice that the total amino acids in feed molasses make up approximately 9% of non-sucroses while in the extract stream 31% of the non-sucroses present are amino acids. Notable examples of amino acids that are concentrated with respect to non-sucroses are serine, alanine, tyrosine, valine, and isoleucine all of which go from under 1%/NS in molasses to 3%/NS or higher in separator extract. Level increases for amino acids, based on non-sucroses, are also shown graphically in Figure 1. Material balance calculations for the individual amino acids give the percent elimination values, that is the percent of entering amino acid that does *not* exit with extract, shown in the last column of Table 2 and graphically in Figure 2. Elimination values range from a high of 94% for glutamic acid to a low of 13% for tyrosine.

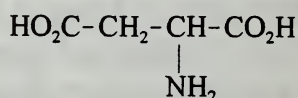
Amino acids as a group are of great practical interest in ion exclusion chromatographic systems because of their overall poor separation characteristics which result in enrichment of extract in some members of the group. Obviously any such poorly separated constituent has the potential to accumulate in factory systems if separator streams are recycled. In addition individual amino acids may cause

specific problems at high levels; tyrosine, for example, is low enough in solubility to crystallize from stored syrups. Another area of interest, and the main topic of this discussion, is the relationship between amino acid properties and separation characteristics.

Amino acid elimination values are somewhat variable but if mean values for three separate tests are plotted versus amino acid isoelectric point the relationship shown in Figure 3 is obtained. Notice that percent elimination is at a minimum for amino acids with isoelectric points in the range of 5.6 to 6.0 while amino acids with isoelectric points outside this range are eliminated more efficiently. This seems to indicate that the ion exclusion system sees amino acids in this range of isoelectric point as small neutral molecules which diffuse into resin beads and travel with sucrose. Composite pH values in the separator are actually higher than this 5.6-6.0 range and amino acids in this isoelectric point range would be expected to be somewhat anionic under separation conditions but there is a pH gradient within such a system and the full effects of pH versus separation characteristics have not been investigated. The amino acids at the extremes of the plot in Figure 3 probably have a higher net ionic charge under separator conditions and are thus excluded from the resin. For example glutamic acid (4) and aspartic acid (5) have low isoelectric points

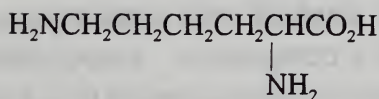


Glutamic Acid
4

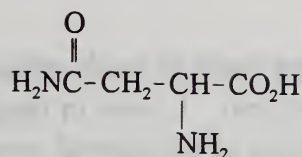


Aspartic Acid
5

due to the second carboxylic acid group present in each molecule. At separator pH levels, far above their isoelectric points, these two species would be predominantly anionic and are always efficiently separated. In contrast, lysine (6) with its high isoelectric point due to a second amine functional group would be expected to be predominantly cationic and has a consistent fairly high elimination value. Asparagine (7) shows a high elimination value even though its isoelectric point is 5.4 but this may be partly due to chemical elimination rather than chromatographic separation. Asparagine can hydrolyze to ammonia and aspartic acid and this may occur to some extent in the separator system. In any event, consistent poor separation of amino acids seems confined to those with isoelectric points between 5.6 and 6.0 probably



Lysine

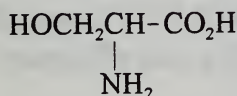
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Asparagine

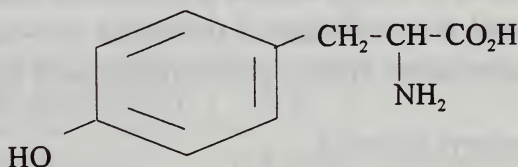
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due to behavior like a neutral molecule as discussed above. The possibility that molecular size also affects separation has been considered but does not seem to be related to elimination values. For example two of the least efficiently eliminated amino acids are serine (8), a 3-carbon amino acid with a molecular weight of only 105, and tyrosine (9), one of the largest amino acid molecules with a molecular weight of 181. These two amino acids with much different molecular sizes but nearly the same isoelectric point behave very similarly in an ion exclusion separation system.

Amino Acid Accumulation in Separation Systems



Serine

8

Tyrosine

9

In addition to their separation characteristics as shown in elimination data, the common amino acids demonstrate another interesting behavior, that of accumulation within a simulated moving bed system. The internal inventory of a component in a separation system at equilibrium conditions can be higher than the level in the feed stream provided that during some period of non-equilibrium operation (such as during start-up) the outflow of the component is less than the amount entering. For example, if parameters are set to hold down the amount of sucrose leaving the system, the internal composite sucrose purity can rise to levels above that of the feed stream. In the system from which the data in Table 2 was collected, the purity of a composite representing all material in the internal separation profile was approximately 67 g sucrose/100 g RDS while the feed molasses was 61 purity. This behavior can also be seen with non-sucrose components and, in fact, several of the amino acids accumulate inside the separation system to a greater extent than any other single component investigated. Table 3 and Figure 4 show, again for the same test as data in Table 2

the level of each amino acid in a composite of all material within the separation system. Levels in feed molasses are shown again for comparison. Also given are ratios of the internal composite amino acid level (based on non-sucroses) to the level in feed molasses. Notice that asparagine and threonine are found at levels of approximately four times that of the feed molasses. Serine and alanine are also present at elevated levels, about twice as high as the feed. Again measured levels vary somewhat from test to test but threonine, serine, and asparagine are consistently greatly elevated in the internal composite while all amino acids except aspartic acid and glutamic acid are consistently elevated to some extent. Total amino acid level in the internal composite is 13.0 g/100 g NS as opposed to 8.9 g/100 NS in feed molasses. Also notice that the level of internal accumulation for a particular amino acid is not necessarily related to percent elimination: tyrosine is eliminated poorly but does not accumulate to extremely high levels while asparagine accumulates but is eliminated well (although chemical reaction is a possible route of partial elimination). Internal accumulation seems to be an indication of how well a component initially fits into the separation profile without totally eluting in either stream rather than a function of which stream it ultimately elutes with. It seems likely, though, that this internal accumulation of particular non-sucrose components must have some effect on separation system performance with respect to system loading and efficiency.

EXPERIMENTAL

All samples were collected over an eight hour period from a factory ion exclusion system processing sugarbeet molasses. Amino acids were converted to phenylthiocarbamyl derivatives by reaction with phenylisothiocyanate^{2,3}. Derivatives were analyzed by HPLC using the Waters Pico TagTM method (triethylamine sodium acetate/60% acetonitrile gradient).

ACKNOWLEDGMENTS

The authors would like to acknowledge the technical support of Stephanie Olmstead and the initial amino acid analysis developmental work of Mary Little.

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Table 1. Nitrogen compounds in sugarbeet molasses (g/100 g non-sucrose).

Betaine	15-17
Total α -amino acids	7-8
Pyrrolidone carboxylic acid (PCA)	8-9
γ -amino butyric acid (GABA)	0.5-2.0

Table 2. Amino acid levels and separator elimination.

Amino Acid	Amino Acid Level				Elimination
	Feed Molasses	Extract	Feed Molasses	Extract	
	g/100 RDS		g/100 NS		g/100 g entering
Aspartic acid	0.461	0.093	1.18	0.952	90
Glutamic acid	0.598	0.071	1.54	0.727	94
Asparagine	0.203	0.104	0.522	1.06	74
Serine	0.274	0.478	0.704	4.38	20
Glycine	0.075	0.104	0.193	1.06	29
γ-aminobutyric acid	0.243	0.247	0.881	2.53	63
Threonine	0.074	0.093	0.190	0.952	35
Alanine	0.215	0.301	0.552	3.08	28
Proline	0.181	0.110	0.465	1.13	69
Tyrosine	0.302	0.510	0.776	5.22	13
Valine	0.178	0.290	0.457	2.97	16
Isoleucine	0.252	0.384	0.647	3.93	22
Leucine	0.228	0.269	0.586	2.75	39
Phenylalanine	0.044	0.022	0.113	0.225	74
Lysine	0.044	0.022	0.113	0.225	75
Total			8.92	31.2	

Table 3. Composite amino acid levels in the separation system.

Amino Acid	Feed Molasses Level	Internal Composite Level	Ratio
	g/100 g NS		Internal Composite /Feed
Aspartic acid	1.18	1.40	1.2
Glutamic acid	1.54	1.28	0.83
Asparagine	0.522	1.86	3.6
Serine	0.704	1.27	1.8
Glycine	0.193	0.276	1.4
γ -aminobutyric acid	0.881	1.13	1.3
Threonine	0.190	0.831	4.4
Alanine	0.552	1.28	2.3
Proline	0.465	0.534	1.2
Tyrosine	0.776	0.870	1.1
Valine	0.457	0.456	1.0
Isoleucine	0.647	0.792	1.2
Leucine	0.586	0.792	1.4
Phenylalanine	0.11	0.14	1.2
Lysine	0.11	0.14	1.2
Total	8.92	13.0	

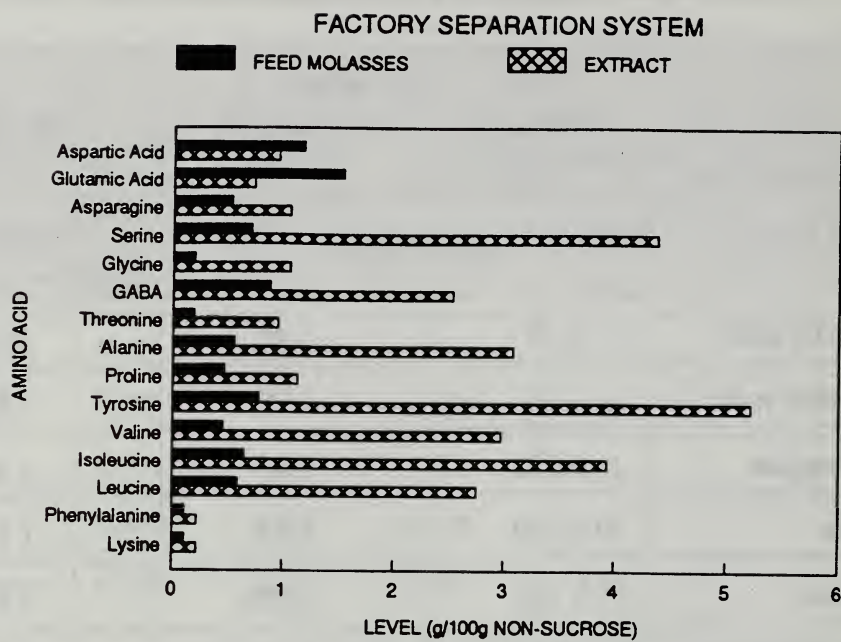


Figure 1. Amino acid levels in molasses and extract.

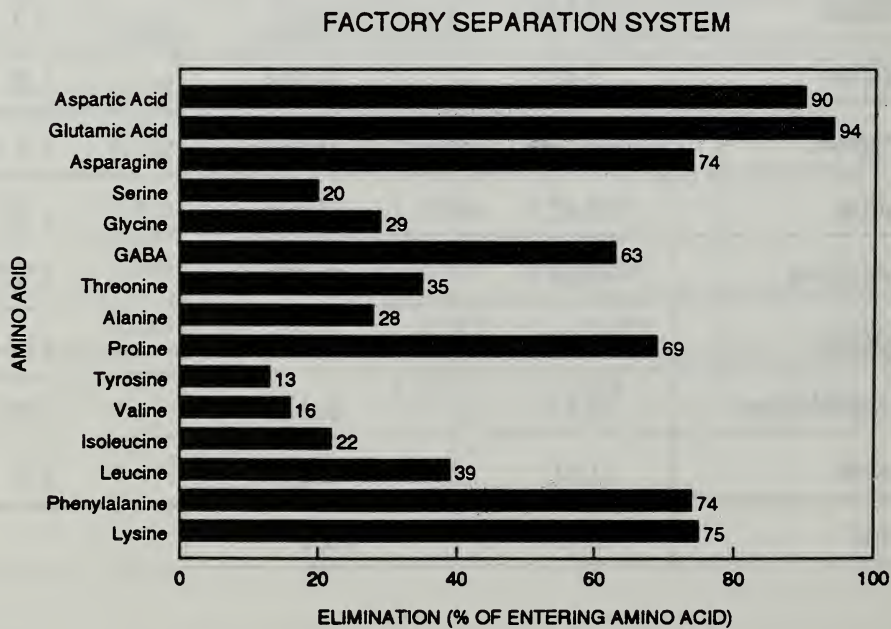


Figure 2. Amino acid elimination values.

ELIMINATION VS ISOELECTRIC POINT
MEAN DATA

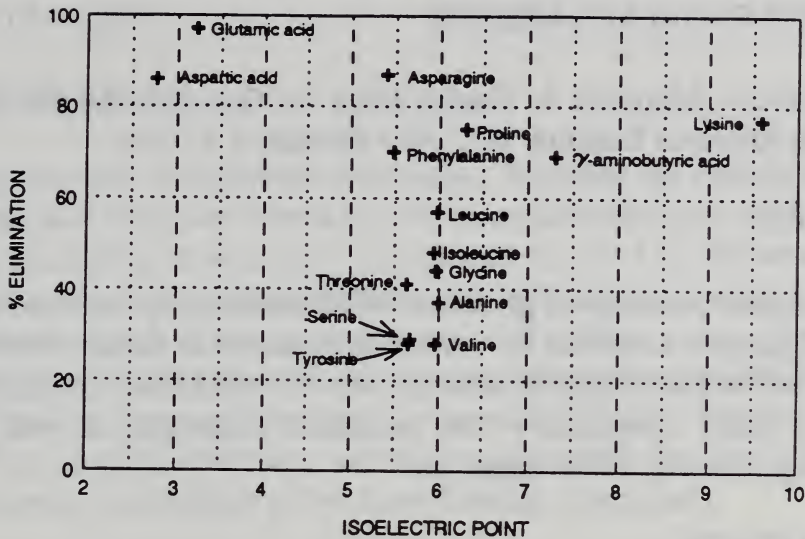


Figure 3. Percent elimination versus isoelectric point.

AMINO ACIDS IN INTERNAL FLUID COMPOSITE
LEVELS AND INTERNAL/FEED RATIOS

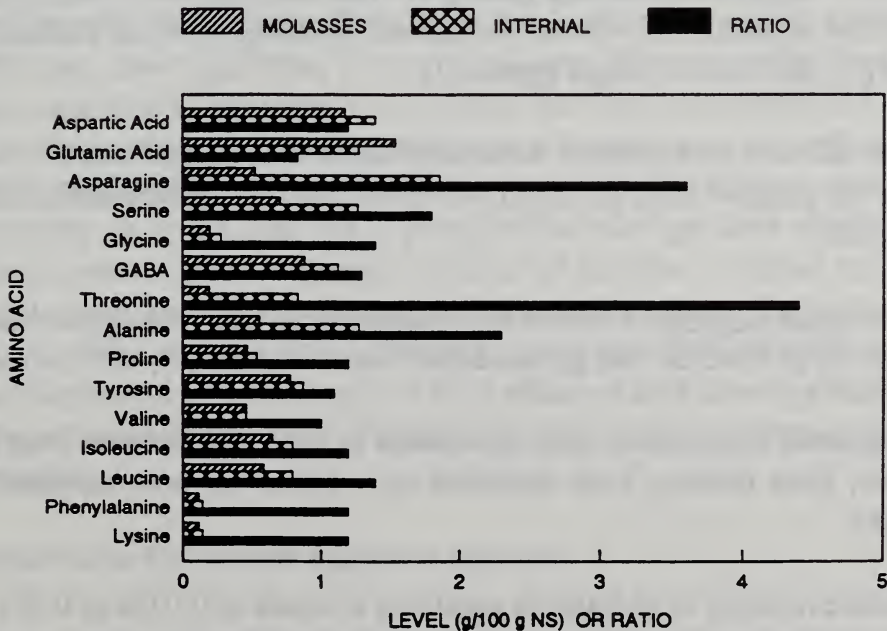


Figure 4. Amino acid internal levels and ratios.

POSTER

SAPONINS FROM SUGARBEET

Earl J. Roberts, Margaret A. Clarke, Mary An Godshall and Les A. Edye, Sugar Processing Research Institute, Inc., New Orleans, LA, USA

ABSTRACT

Several saponins, isomers of glycerides of oleanolic acid, are reported in sugarbeet (*Beta vulgaris*). Isolation by several procedures is reported here. Structural identification of the various species by GC-MS and HPLC is described. Yield of saponin(s) varies considerably with extraction technique, as well as with origin (variety, age, climate) of the sugarbeet.

INTRODUCTION

Saponins are a class of compounds widely distributed in the plant kingdom in legumes, roots, shrubs and bushes, in varying degrees of concentration. Various saponins have been used as soaps because of their surface active properties - hence their name. Saponin-containing plants, or their extracts, have been used in herbal medicine, in treatment of various complaints including liver and cholesterol related diseases (3), and as anti-fungal agents (2).

Saponins fall into two classes: triterpene-based and steroid-based. To the base aglycone are attached sugar group(s) and glucuronic acid, which define the molecule as a saponin.

Sugarbeet (*Beta vulgaris*) is known to contain at least three triterpene-based saponin structures, all glucuronic acid glycosides of oleanolic acid (5), as shown in Figure 1.

Two additional compounds, seco-glycosides of saponins, isolated from beet leaves and roots, have recently been identified (4). Up to six beet saponins have been postulated.

Saponins are reported to be found in sugarbeet at levels of 0.01% to 0.2% of beet (2, 10, 11), and at less than 100 ppm, generally less than 20 ppm, in white sugar. Saponins are most densely concentrated just under the sugarbeet skin, where they

function as plant defense compounds, against disease and against frost damage, and are located in cell membranes (2). They are most highly concentrated in small beets grown in warm climates.

REASONS FOR STUDY

1. Foaming problems in sugarbeet processing. Saponins are surface active agents in general, and have often been blamed as the cause for foaming in juice purification, evaporation and boiling in beet sugar manufacture (2, 10, 11). However, in several trials whereby saponin, or saponin extract, was added to sugar solution and foam formation measured, lack of foaming was observed (2). The current authors speculate that the diversity of results in foam formation, and also in floc formation (see below) may be ascribed to the procedures used for saponin isolation. Precipitation in acid, about pH 1.5, is usually the first step, and this procedure will also precipitate peptides, which are known to be surface active foam-forming compounds.

2. Floc problems in beet sugars. Acid beverage floc, which can form in sugar-sweetened carbonated soft drinks after several days standing, has been ascribed to both beet and cane sugars. In general, any haze or turbidity in a soft drink is referred to as "floc", but there are specific characteristics which define acid beverage floc, most notably that it disappears upon shaking. Beet and cane flocs can appear as a hazy turbidity or as fluffy masses. Beet sugar floc is more granular in appearance and less fluffy than cane sugar floc, in our experience. Beet sugar floc has, for many years, been ascribed to saponins (1, 11), but in our tests, the addition of amounts of isolated saponin added at levels resembling those in sugar to a sugar floc test do not necessarily produce floc. The literature supports this: Eis (1) says that "separated floc" (actually all strong acid, pH 2, precipitates) can "produce effervescence and flocculation when sufficient neutral solution of the floc is added to carbonated beverages". In the authors' experience, "sufficient" is far above the levels of saponin reported in white sugars (<1 to 30 ppm). It is therefore of interest to isolate sugarbeet saponins for further study of their effect on acid beverage floc formation: the evidence for the responsibility of sugarbeet saponins for this phenomenon may be circumstantial.

3. Potential uses for isolated sugarbeet saponins.

EXTRACTION OF SAPONINS

1. Rother's aqueous extraction. Fresh beet peelings (5.5 kg) were covered with water in a blender and divided into small pieces. The slurry was heated to 90°, and filtered on fabric. The residue was suspended in water, heated, and filtered again on fabric. The pH of the filtrate was adjusted to 1.5 with HCl, heated to 90°C for one hour, and allowed to settle overnight. After settling, the supernatant liquid was decanted. The residue was mixed with filter aid and filtered; that residue was washed with water, adjusted to pH 1.5 with HCl, and allowed to air dry. The dried residue was extracted in a Soxhlet extractor with ethanol, the ethanol solution concentrated, and poured into water at pH 1.5. The precipitate was dissolved in hot ethanol and again precipitated by pouring into pH 1.5 water. The precipitate was filtered off on hardened paper, dissolved in water, and evaporated to dryness at low temperature; yield 3.0 g of brown material (9).

Analysis of this material by TLC (as described below) showed oleanolic acid (the aglycone, or saponin) and nothing corresponding to saponins. Mass spectroscopy analysis confirmed the presence of oleanolic acid. Apparently the harsh acidic treatment hydrolyzed the saponins, leaving only oleanolic acid in the isolation.

2. Aqueous extraction, Ridout et al (5). On another experiment 1734 g of beet peel was ground in a blender. The slurry was filtered on fabric and the residue mashed with water. The pH of the filtrate was adjusted to 1.5 with HCl, heated to 85°C for 15 minutes, cooled overnight, and filtered on fabric coated with filter aid. The filtrate was returned to the filter twice more and the residue was washed with warm 1N HCl. All filtrates were discarded. The filter was then washed with warm 2N NaOH solution until the filtrate was clear. The filtrate was placed in a large beaker and HCl was added to reduce the pH to 1.5. The precipitate was collected on fabric coated with filter aid as before, washed with 1N HCl, and the filtrate discarded. The filter was then washed with warm 2N NaOH. The filtrate was acidified to pH 1.5 with HCl, filtered through Whatman 542 paper, washed with water, and extracted with 500 ml of warm ethanol. The filtrate was evaporated to dryness, then taken up in water and freeze dried, yielding 2.0 g of brown material. TLC analysis showed oleanolic acid but no saponin.

3. Methanol extraction, Ridout et al (5). Freeze dried beet peel (650 g) was crumbled into small pieces and extracted in a Soxhlet extractor with methanol. The

methanol was evaporated under reduced pressure, residue dissolved in water, and extracted several times with 1-butanol.

The butanol was evaporated and the residue was dissolved in water and dialyzed against flowing tap water in a 12,000 MW cut off bag for 24 hours. The material remaining in the bag was filtered, concentrated, and freeze dried. Yield, 5.6 g of cream colored material. This material was subjected to thin-layer chromatography and GC-MS analysis.

COMPOSITION AND STRUCTURE OF SAPONIN BEET ISOLATES

Saponins are known to exist in variety in any one plant - a single structure is not common. Variations in the sugar moiety structure and linkage position are observed. Sugarbeet saponins are no exception. The three forms shown in Figure 1 all have as their base unit oleanolic acid, a carboxylic acid triterpene.

Isolates from aqueous extraction. Isolates prepared by the traditional aqueous extraction methods (1 and 2), with repeated extractions at pH 1.5 and washing with base, showed only oleanolic acid in the final dried extract, and no saponins. Oleanolic acid identification on thin layer chromatography (solvent system: chloroform; methanol; water 65:35:10), made visible by 2N H₂SO₄, or anisaldehyde spray, was confirmed by GC-Mass Spectrometry identification, as shown in Figure 2.

Method 1, of Rother (9), using aqueous extraction and low pH, yielded 3g (0.05% on beet peel) brown solids; Method 2, of Ridout et al (5), yielded 2 g (0.12% on beet peel), of brown material. Method 3 of Ridout et al (5), similar to that of Ireland (3) using methanol extraction and not including low pH treatment, yielded 5.6 g (.8% on beet peel) of cream colored material. Thin layer chromatography of the methanol extracted material showed five major components, two of which traveled with an authentic saponin (probably soybean) obtained from Sigma Chemical Co. It should be noted that "saponin extracts" supplied to S.P.R.I., Inc., by several sugar companies (sponsoring companies of S.P.R.I., Inc.) appeared to consist mainly of oleanolic acid.

RESULTS AND DISCUSSION

It is evident from comparison of the aqueous methods of extraction with the methanolic method that the sugarbeet saponins are indeed present in the whole root,

in the peel, just under the skin. It is also evident, from chromatographic and mass spectroscopic data, that the saponins extracted by aqueous methods have been hydrolyzed by the strong acid treatment so that only the aglycone (or sapogenin), oleanolic acid, remains.

This observation throws some doubt on earlier work, all of which isolated saponins by aqueous extraction with strong acid treatment. Were these earlier results the product of oleanolic acid only? Earlier workers did not have the benefits of GC-MS, but had to rely on colorimetric tests, which may give a false positive for saponins when oleanolic acid is present.

Recent work (5) found saponins by aqueous extraction, not in extracts of beet roots, but only in extracts from beet molasses, where the compounds may be expected to concentrate.

Results are discussed from the point of view of reasons for this work.

BEVERAGE FLOC

Floc tests (50 Brix, phosphoric acid to pH 2) were run on non-floccing sugars with the addition of varying amounts of beet extract, or commercial saponin (not beet), or oleanolic acid. Saponin and oleanolic acid were also tried in combination with protein (gelatin and α -amylase were used). The methanolic extract of beet root formed a floc, as did the combination of oleanolic acid and protein.

The observation that saponins apparently are hydrolyzed during the extraction raises a basic question about reactions in and causes of floc formation. The assumption was that floc material was acid insoluble, and therefore the aqueous extraction method at low pH was developed. But do saponins themselves, if in white sugar and therefore in beverage, become hydrolyzed at beverage pH (about 2 - 2.5)? In that case oleanolic acid and not saponin would be responsible for floc formation.

In studies reported here, the only floc former known to contain whole saponin was the methanolic extract, i.e., the extract that contains unhydrolyzed saponins. So it would appear that whole saponin - or at least whole when it enters the beverage - does form beverage floc.

The observations on hydrolysis explains why Eis (1) found it necessary to add back a relatively large quantity of isolated floc material before observing flocculation; probably sufficient was added back to form a haze rather than a true floc.

The authors have, in past work (6, 7, 8), observed that isolated beet sugar floc (from beverage), identified as beet sugar-sourced by the high level of raffinose present, contain beet cell wall polysaccharide with galacturonic acid residues and protein. The polysaccharide (given the trivial name Indigenous Beet Polysaccharide, IBP) is comparable to the cell wall sugarcane polysaccharide, containing glucuronic acid groups, that can cause acid beverage floc when in combination with a protein. At beverage pH, the acid groups become negatively charged, the protein groups become positively charged; charge attraction brings the molecules together to form first a coacervate and then a flocculating network that entraps colloidal and suspended material to form a visible floc.

The authors propose that a similar mechanism is responsible for beet sugar floc: a carboxylic-acid containing molecule, cell wall polysaccharide or saponin, becomes negatively charged (carboxylate ion formation) at low pH; an amino-group containing molecule (protein, peptide, or other) becomes positively charged. The two come together from charge attraction to initiate a floc network. This explanation accounts for the observance of floc without saponin present because another negatively charged molecule can be participating (perhaps oleanolic acid). Both saponin and oleanolic acid contain a glucuronic acid group. It accounts for the presence of saponin without floc, if insufficient protein or positively charged amino group is present, and also for the presence of floc without saponins.

SAPONIN TESTS

All these postulates and observations involve validity of saponin tests. The traditional tests must be re-evaluated using instrumental analysis to distinguish between saponin and oleanolic acid.

FOAM PROBLEMS

The confusion in saponin and sapogenin isolates - usually aqueous isolates for foam testing - requires re-evaluation of the foam problem causes and effects.

CONCLUSIONS

Comparison of traditional aqueous acid extracts of "saponin" from sugarbeet substrates with methanol extraction has shown that aqueous extraction yields only the aglycone of beet saponins, oleanolic acid. Chromatographic and mass spectrometric evidence support this.

A re-examination of the relationship between saponin and oleanolic acid and "saponin" - caused problems, acid beverage floc and foaming, is required.

It is proposed that floc formation has two causative factors: presence of a molecule that is negatively charged at beverage pH (saponin, oleanolic acid, or cell wall polysaccharide) and a molecule that is positively charged at low pH (protein or peptide); the two molecules come together in solution through charge attraction to form a coacervate that develops into a floc network.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the kind contributions of isolates containing saponin from several sponsoring companies of S.P.R.I., Inc.

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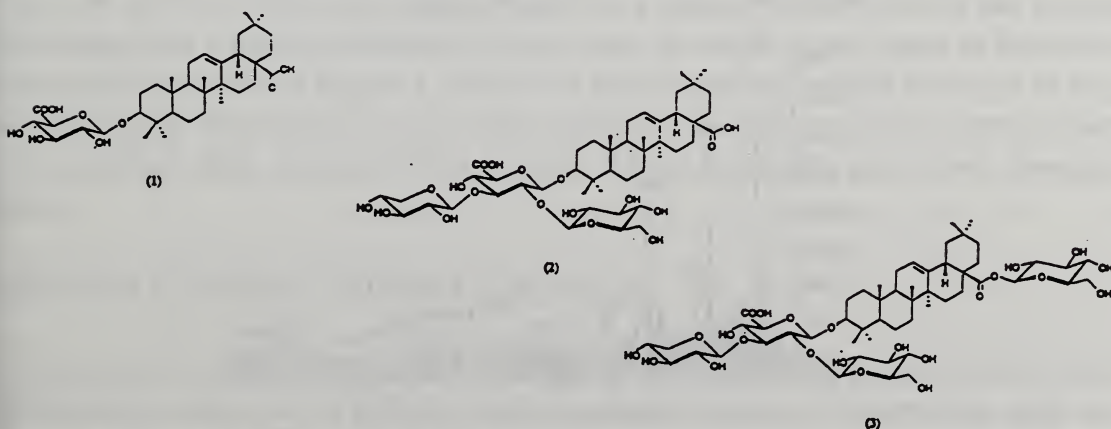
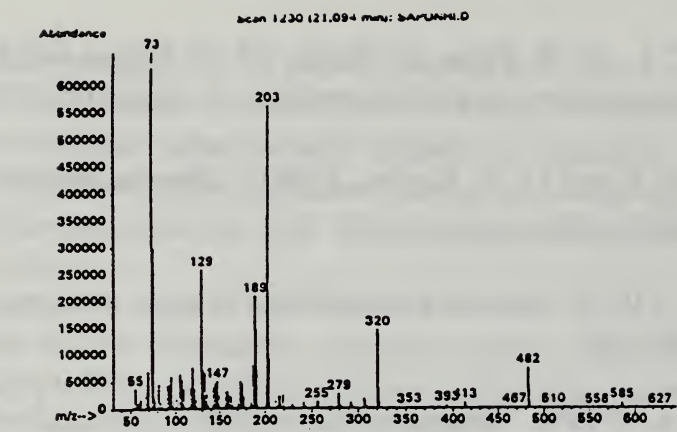
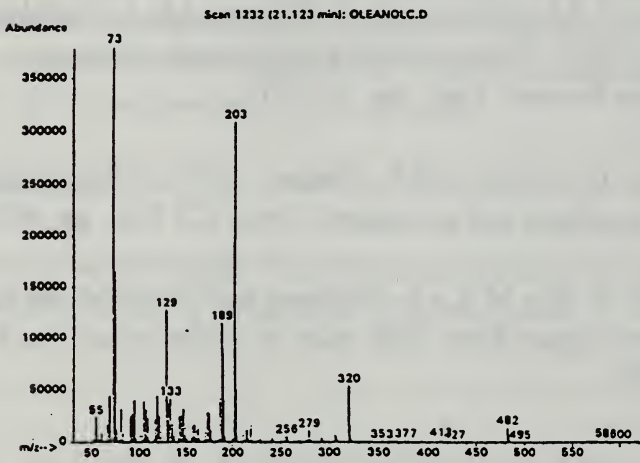


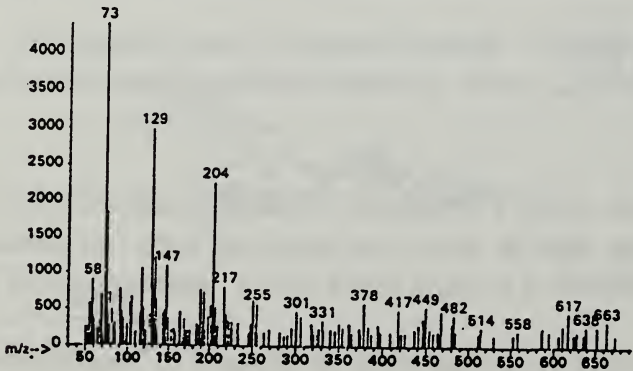
Figure 1. Three saponins of sugarbeet (5).



Mass spectrum showing oleanolic acid in aqueous extract of beet peel.



Mass spectrum of authentic oleanolic acid TMS. Match quality with beet peel extract is 99%.



Mass spectrum of methanolic extract of beet peel, showing absence of oleanolic acid.

Figure 2. Mass spectrometric analysis of sugarbeet extracts.

POSTER

IDENTIFICATION AND DETERMINATION OF CARBOHYDRATES USING INSTRUMENTAL PLANAR CHROMATOGRAPHY

Giuseppe Vaccari, Gaetano Lodi and Elisabetta Dosi, University of Ferrara, Ferrara, Italy

INTRODUCTION

Although planar chromatography was the first chromatographic technique to be utilized for the separation of sugars, even in complex matrixes, it was later replaced by GC and HPLC. The reason was that for the latter techniques increasingly sophisticated instruments were developed and these did not apply to planar chromatography.

Recently instrumental TLC techniques have been put on the market which are very much improved compared with earlier methods and these are completely automated. Such techniques cover all the various phases of analysis, starting from placing the samples on the plate to elution, then development and finally the qualitative/quantitative evaluation. Moreover, layers with high performances (HPTLC) are now available which significantly improve the separation efficiency.

THE NEW HPTLC LAYERS

The new HPTLC layers are characterized by a narrow distribution of the particle dimensions and a marked decrease in their sizes, as can be clearly seen in the microphotographs shown in Figure 1. Such new layers need very small amounts of sample and shorter migration distances, thus making it possible to achieve great separation potential, time saving for the analysis, better resolution and lower detection limits.

MODERN PLANAR CHROMATOGRAPHY

As well as the utilization of layers having high performances, modern planar chromatography makes use of efficient and automated systems of chromatographic elution, as shown in Figure 2. In the present paper concerning the separation and determination of carbohydrates in complex matrixes, we deal with HPTLC using

the AMD development method (see below). The completely automated densitometric technique was used for quantitative determination.

THE TECHNIQUE OF APPLYING THE SAMPLE

The samples to be analyzed, which in general need to be simply diluted, must be placed on the layer as small diameter spots or as sufficiently thin bands such as not to influence the efficiency of the layer. The amount of sample must not be too great (a few microliters) to avoid volume or mass overloading. Moreover, a known amount of sample must be placed on a precise point. Various automatic devices are available which allow the deposition of spots or bands having the characteristics mentioned above. One such device, which is normally utilized mechanically, moves the plate beneath a fixed syringe according to a fixed programme. A nitrogen flow sprays the sample from the syringe through an atomizer thus giving a very thin band on the plate surface. In the densitometric determination the thin bands are easily located on the plate.

The reproducibility of the volume of the applied sample is very high and the whole operation takes only a few minutes.

THE TECHNIQUE OF AUTOMATED MULTIPLE DEVELOPMENT (AMD)

The most efficient method of increasing the detection under capillary flow conditions is based on multiple development (1). For this the TLC plate is developed over a certain distance and time. Then the development is stopped and the solvent removed from the plate by evaporation. The development process is then automatically repeated a number of times. This strategy for the separation of complex mixtures allows for variations of the primary experimental parameters: the length of the chromatographic plate, the composition of the mobile phase in each development and number of developments. Another particular characteristic of multiple development is the mechanism of reconcentration of the zone, so increasing the efficiency of the layer by limiting the diffusion of the zone. In order to increase the reconcentration effect it is necessary that the number of steps is sufficiently great (at least ten) which makes it absolutely necessary to automate the whole process. Now the process of the automated multiple development (AMD) is feasible by utilizing instruments whose mode of operation is depicted in Figure 3. For the separation of sugars we used the CAMAG AMD instrument (Muttentz, Switzerland). The AMD operating sequence begins with nitrogen drying which involves the evacuation of

the sealed developing chamber. This is followed by a conditioning step to control layer activity. To commence development, a mixing valve selects the initial solvent composition from the solvents available in the mixing chamber. At the appropriate time, a fixed mobile phase volume is forced into the developing chamber and a chromatographic separation developed over a preselected distance. The mobile phase is then sucked from the developing chamber into a waste solvent bottle and the solvent vapours evacuated from the chamber by means of a vacuum pump. After the drying step, the next cycle commences by conditioning the layer, then introducing a fresh mobile phase, and subsequently removing it at the end of the selected development time and then again drying the layer under vacuum. All the processes are automated and time-sequenced by a program introduced into the control unit and it can be actuated without operator intervention. Depending upon the scheduled number of developments, the whole cycle can proceed for several hours and can even be followed overnight because of the complete automation of the system.

DERIVATIZATION

After AMD development, depending upon the characteristics of the layer, the plates can be derivatized by direct heating on a hot plate for a few minutes or be previously treated with an appropriate reagent and then placed in an oven for a few minutes. Even the derivatization step can be carried out via an automated system in a few seconds using a simple device which dips the plate into the derivatization solution.

QUANTITATIVE DETERMINATION VIA DENSITOMETRY

Thin layer chromatography would not be able to compete with other chromatographic techniques without the availability of instruments capable of quantifying the planar separation in situ. At the present time, suitable scanners are marketed which make it possible to translate in few minutes the results of a separation on a plate into absorption spectra and to give a quantitative evaluation of the separated components. We actually used a CAMAG - SCANNER III interfaced with a computer and managed by CATS 4.03 software. In the specific case of the determination of sugars, the plates were scanned for absorbance (`{SIMBOLO 108 \f "Symbol"}` max = 515 nm).

RESULTS

After a series of preliminary tests (2) the following operational conditions were selected:

- 1) Type of plate: HPTLC-NH₂ (Merck, Darmstadt, Germany).
- 2) Gradient conditions: each plate was eluted in 15 steps using acetonitrile-acetone-water mixtures, the composition of which progressively changed from an initial 1:1:1.6 ratio to a final 2:2:1 ratio.
- 3) Analysis of cane and beet molasses: molasses samples were dissolved in water (0.5g/100ml) and the solution so obtained was diluted 1:1 with water-acetone mixture (1:1); such a solution was directly applied to the plate (2 {SIMBOLO 109 \f "Symbol"}1) without any purification treatment. For quantitative determination it was also necessary to apply to the plate standard solutions containing known concentrations of the various sugars to be determined. After applying the spots and the AMD elution, the plate was derivatized with an {SIMBOLO 97 \f "Symbol"}-naphthol solution (3). After 5 minutes heating in an oven at a temperature of 110°C the scanner reads the plate and registers the various chromatographic separations which makes possible a qualitative/quantitative evaluation of the various components. Preliminary calibration tests demonstrated that the correlation coefficient for the various sugars is always higher than 0.99 and the standard error for the quantitative determination varies from 1% for the components present in greater amount to 10% for those present at lower concentrations. Figures 4, 5 and 6 show the densitograms for a mixture of standard sugars, and for cane molasses and beet molasses, respectively.

CONCLUSIONS

The HPTLC-AMD technique represents a valid alternative to the analytical techniques at present in use for the determinatin of sugars in complex matrixes. In fact, as well as the simplicity and the complete automation of the system we can stress that the sample only has to be diluted and many samples can be analyzed simultaneously. Thus, both the specific cost and the time taken for the analysis are very limited in comparison with other analytical methods. Even the total cost of the whole instrumentation can be favourably compared with the cost of alternative analytical devices. Employment of HPTLC-AMD makes possible not only the evalua-

tion of the characteristics of the final products in sugar processing but also to follow the composition of the various juices during such processing. Moreover, this analytical technique is very versatile because we can even evaluate the composition of the so-called non-sugars. In particular it is applicable to the determination of colorants and colorant precursors, such as flavonoids and polyphenols, by modifying the type of layer, the composition and type of gradient, as well as, the detection technique.

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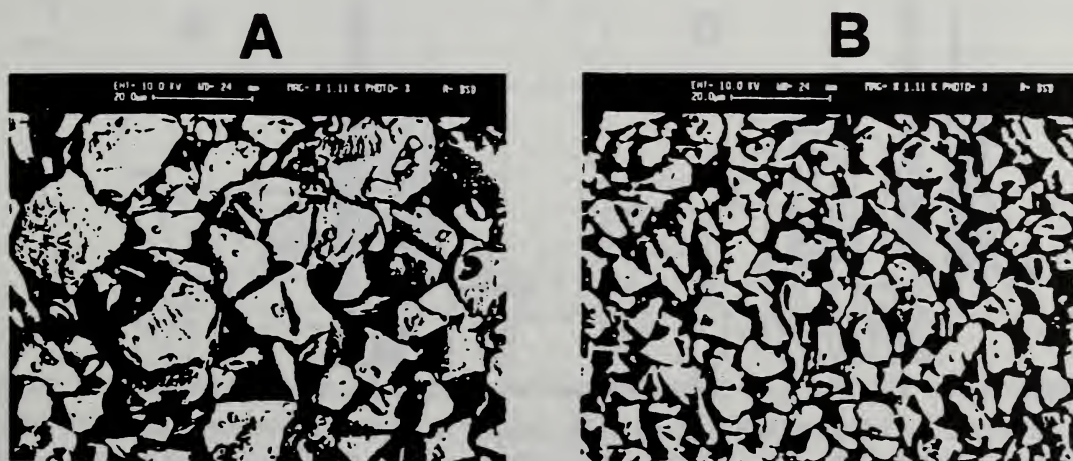


Figure 1. Microphotographic comparison between traditional TLC plates (A) and HPTLC plates (B).

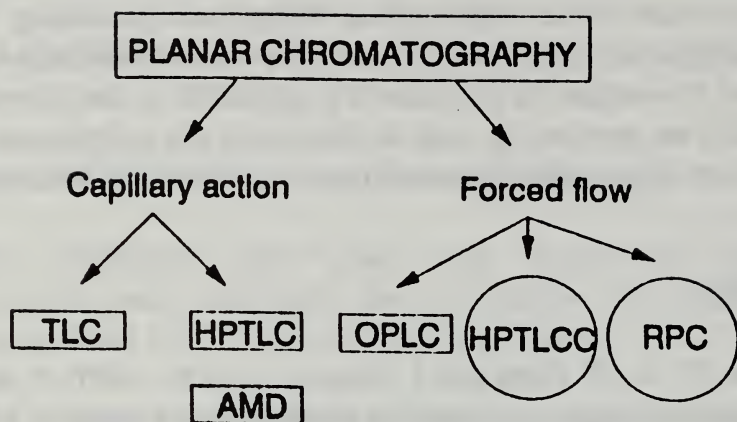


Figure 2. Different elution methods in planar chromatography. TLC: Thin Layer Chromatography; HPTLC: High Performance Thin Layer Chromatography; OPLC: Over Pressure Layer Chromatography; HPTLCC: High Performance Thin Layer Circular Chromatography; RPC: Rotational Planar Chromatography; AMD: Automated Multiple Development.

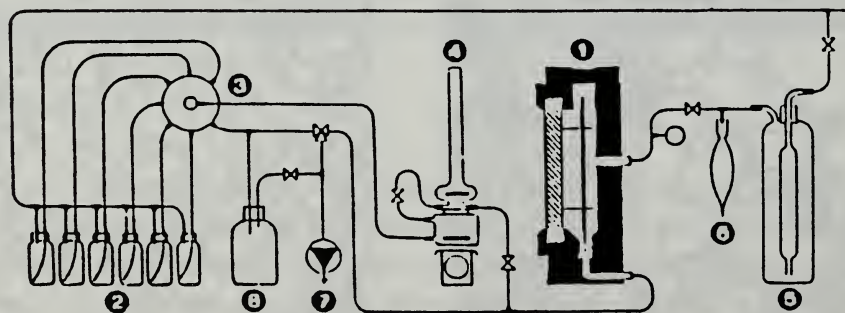


Figure 3. Scheme of the CAMAG-AMD instrument. 1) Developing chamber; 2) Reservoir bottles; 3) Valve; 4) Gradient mixer; 5) Wash bottle; 6) Reservoir; 7) Vacuum pump; 8) Waste collecting bottle.

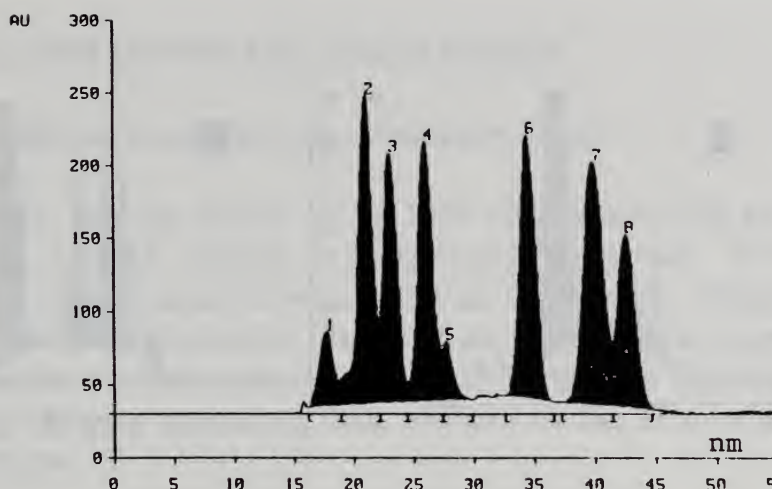


Figure 4. Separation of a standard mixture of sugars: 1) Fructosyl nystose (GF4); 2) Nystose (GF3); 3) Raffinose; 4) 1-Kestose; 5) 6-Kestose; 6) Sucrose; 7) Glucose; 8) Fructose.

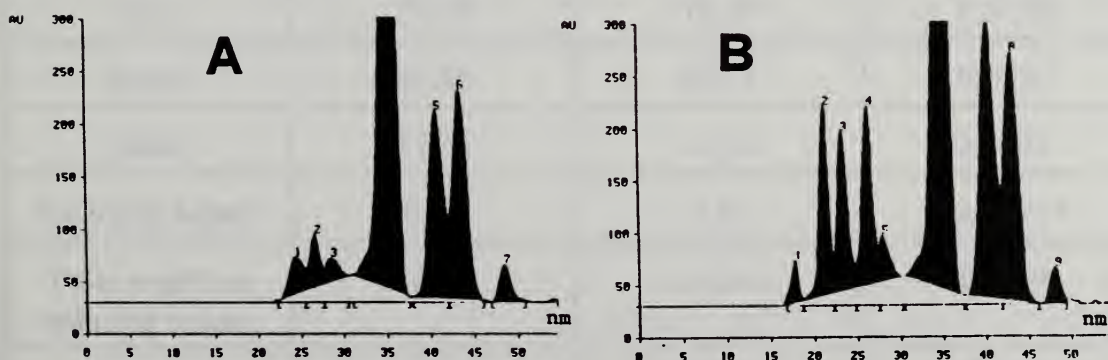


Figure 5. Chromatogram of a cane molasses sample (A) and the same sample added with the standard solution of sugars (B). (A) 1) Raffinose; 2) 1-Kestose; 3) 6-Kestose; 4) Sucrose; 5) Glucose; 6) Fructose; 7) unknown; (B) 1) GF4; 2) GF3; 3) Raffinose; 4) 1-Kestose; 5) 6-Kestose; 6) Sucrose; 7) Glucose; 8) Fructose; 9) unknown.

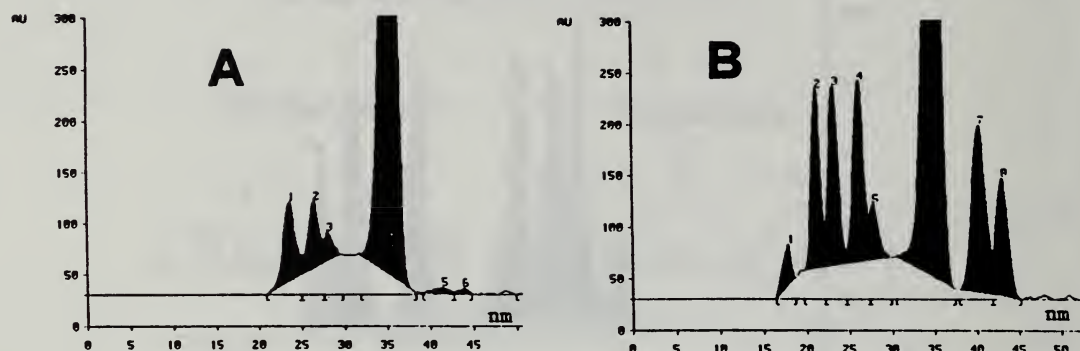


Figure 6. Chromatogram of a beet molasses sample (A) and the same sample added with the standard solution of sugars (B). (A) 1) Raffinose; 2) 1-Kestose; 3) 6-Kestose; 4) Sucrose; 5) Glucose; 6) Fructose. (B) 1) GF4; 2) GF3; 3) Raffinose; 4) 1-Kestose; 5) 6-Kestose; 6) Sucrose; 7) Glucose; 8) Fructose.

POSTER

NIR ANALYSIS OF SUGARCANE IN BRAZIL

Henrique Amorim, Fermentec Ltda., Piracicaba, Brazil

At the factory, Vale Do Rosario, in the 1995-1996 season, NIR analysis was run in parallel with standard methods on finely chopped sugarcane. Cane samples were taken at the Core sampler laboratory at Vale Do Rosario. Samples were further chopped up in a model shredder (South African design). Two sample preparations were employed: (1) the standard press method and (2) a digester method (water and heat). Results comparing NIR analysis of the finely shredded cane are presented below.

NIRSYSTEMS ANALYSIS COMPARED TO CONVENTIONAL ANALYSIS

Vale Do Rosario - 1995

Cane - Press method (on solids)			
Analysis	Lab	NIRS	Prob > T
Brix	18,99	19,03	0,4164
Pol	15,75	15,79	0,3170
Purity	82,89	82,93	0,6397
Fiber	12,43	12,52	0,0001*
Reducing sugars	0,83	0,93	0,0001*
Total sugars as reducing sugars	16,87	16,91	0,7391
Premium	27,17	27,21	0,7249

Average of 1,803 analyses

Vale Do Rosario - 1995

Cane - Digester method			
Analysis	Lab	NIRS	Prob > T
Brix	21,03	21,31	0,0001*
Pol	18,19	18,29	0,0001*
Purity	86,83	86,89	0,3029
Fiber	13,74	13,99	0,0001*
Pol % cane	15,02	15,13	0,5292
Premium	26,57	26,69	0,0254

Average of 409 analyses

POSTER

HPLC ANALYSIS IN THE SUGAR INDUSTRY IN COSTA RICA

Lourdes Quesada, Consultores Asociados Lourdes Quesada, San José, Costa Rica

INTRODUCTION

Costa Rica is a country in Central America with a population of almost three million people. Its economy is based on agriculture: coffee and bananas are the main products for export to Europe and North America. However, Costa Rica has had plantations of sugarcane for more than 50 years, with 113,750 acres in different areas of the country in cane. Some varieties have been brought from other countries while others have been developed in our research laboratories, with biological control included.

The owners of the factories and the cane growers formed an association 50 years ago, for mutual purposes. This office, called "Liga de la Caña", LAICA, is in charge of dealing with the local and external market for sugar, following changes in the worldwide market. LAICA controls the quality of the sugar produced in Costa Rica through its laboratory. In 1994, LAICA bought HPLC equipment to do some analyses for the factories and field, to compare with the traditional methods (polarization and Brix) and do some research. This instrument was used for trials in several different companies. Since then, after the first year, three sugar mills bought their own HPLC equipment to continue the work. This brief paper contains some preliminary results of this work.

EXPERIMENTAL

The High Performance Liquid Chromatography instruments are Hewlett-Packard and Perkin-Elmer, with an isocratic pump, column heater and refractive index detection. The analysis is run at column temperature 85°C, flow rate 0.60 ml/min, with water distilled, deionized and filtered (0.45 microns) as mobile phase. Average pressure for a run is 500 psi. The sample is prepared by filtration and centrifugation, and finally is filtered through a 0.22 μ Millipore type filter. Sometimes I believe this preparation is not enough when starch is present.

RESULTS AND DISCUSSION

We analyze different kinds of samples: press cane, primary and mixed juice, molasses, etc., with emphasis on the juices. Studies have confirmed that is very important to reduce the time between when the cane is cut and when it is milled: no longer than 24 hours is best. It is very important to maintain these conditions if the cane was treated with ripeners, and specially if it is more than six weeks after application. The behavior of the content of invert sugar in treated sugarcane can be compared with the sugarcane without ripeners. Some studies show that some cane varieties produce more sugar but deteriorate faster than others.

We determined that final molasses has a greater concentration of fructose than of glucose. When results of apparent purity are extremely low, this is caused by many factors but especially by the optical rotation of the fructose in comparison with that of sucrose and glucose. Fructose has a negative optical rotation, therefore polarization is low. Low purity is not real and not a good assessment of sugar produced. This could result from two causes: from cane with high invert sugars entering the factory, and from the invert produced by the factory. The last one is easy to control with proper cleaning and chemical control. To find the best product quality, HPLC can be used to monitor the production of invert very easily and fast.

Future work will include studies in other areas like starch and dextran, low and high molecular weight, in sugarcane.

POSTER

MEMBRANE FILTRATION APPLICATIONS IN THE SWEETENER INDUSTRIES

John Juszczak, Niro Hudson, Inc., Hudson, Wisconsin, USA

Niro's Filtration Division, considering its roots in the DDS (Der Danske Sukkerfabrikker) membrane filtration group, has been involved with membrane filtration in the sweetener industries since the 1960's. Relatively recent developments in membrane technology make applications viable today that were only dreamed about in those early days. This poster presentation outlines some of the various membrane applications that have been proposed for the cane industry. The poster will touch upon some of the work done by Niro on purification of low purity refinery syrups.

The poster will also present an overview of work conducted in the beet sugar and dextrose industries. Dextrose, in particular, provides a good case study for the cane industry as long term operation of commercial systems has demonstrated the value of the technology. Niro began work with the dextrose industry over eight years ago and our first commercial dextrose system has been on-line for four years. Figure 1 outlines the size ranges of the common technologies, and their abbreviations.

Proposed cane sugar membrane applications in sugar mills:

- Purification of clarified cane juice (MF/UF)
- Purification of cane syrup (MF/UF) (see Figure 4)
- Purification of molasses (MF/UF)
- Purification of raw sugar (MF/UF)
- Concentration of clarified juice (NF/RO)
- Recycle of evaporator condensate (RO)

Proposed cane sugar membrane applications in refineries:

- Purification of low purity syrups (MF/UF) (see Figures 2 and 3)
- Purification of melt liquor (MF/UF)
- Recycle of ion exchange brine (NF)

SPRI

- Recycle of char washwater (NF/RO)
- Recycle of evaporator condensate (RO)

Commercial membrane applications on sugar syrups

Cane sugar industry:

- Clarification/purification of cane juice

Beet sugar industry:

- Clarification/purification of sugar beet juice

Dextrose industry:

- Clarification/purification of dextrose syrup (95 DE, ~30° Brix)
- Clarification/purification of malto-dextrose syrups (low DE < ~30° Brix)
- Clarification/purification of HFCS (high fructose corn syrup, ~30° Brix)
- Depyrogenation of IV dextrose (intravenous dextrose, ~30° Brix)

Basic membrane configurations

Spiral wound: These membranes offer the highest packing density and energy efficiency. Sensitive to suspended solids loadings and especially to fibers. Fairly good viscosity capability with properly designed modules. Available in MF, UF, NF and RO membranes and in a variety of diameters. Currently being used commercially on dextrose syrups.

Tubular: Much lower packing density and energy efficiency but very good suspended solids and viscosity capabilities. Available in a variety of tubule diameters. Available in MF, UF, NF and RO membranes.

Ceramics: Best thought of as small bore tubular membranes. Membrane has high energy consumption but better productivity than corresponding polymeric membranes. Capital cost is generally higher than polymeric but life is generally MUCH longer. Available in a variety of flow channel diameters; available in MF and UF membranes. Currently in use commercially on dextrose syrups and cane juice.

Hollow fiber: These are best thought of as very fine bore tubular membranes. These can vary in diameter from the size of a hair up to about 1/8" diameter. These have a good membrane packing density and a fair energy efficiency. All but the largest diameters have very limited suspended solids, fiber and viscosity capabilities. Many types of hollow fibers have a reputation of being delicate and easily broken by over-pressuring or pressure surges. These are available in MF, UF, NF and RO membranes. Currently in use commercially on sugarbeet juice.

Plate and frame: These are intermediate in packing density and energy consumption between spirals and tubes. They have fairly good suspended solids and fiber capability and very good viscosity capability. Membrane replacement can be tedious and time consuming. These are available in MF, UF, NF and RO. Much of the early experimentation was conducted on this equipment.

Benefits of membrane filtration on low purity refinery syrups:

- Large reductions in starch, dextran and other polysaccharides
- Greatly increased syrup clarity (reduction in turbidity)
- Reduction in syrup color
- Reduction in syrup viscosity
- Removal of microorganisms (bacteria, yeast, mold, spores, etc.)
- Removal of suspended solids
- Increased sugar yield from processing

Benefits of ceramic membranes:

- Very long membrane life: often multiples of polymeric membranes
- Very robust membrane: very resistant to damage from pressure swings or other process upsets
- Very chemically resistant: may be cleaned with very high or very low pH or with strong oxidizing agents such as hypochlorite (bleach)
- Hydrophilic membrane: membrane resists fouling from many organic compounds including waxy materials
- "Backpulsable": this technique can often be used to improve productivity and flux stability to maximize time between cleanings

Documented benefits of membrane filtration in dextrose processing (vs rotary vacuum filters) (95DE syrup, ~30° Brix):

- Increased syrup clarity
- Reduction in higher polysaccharides
- Better performance of downstream ion exchange and immobilized enzyme columns (reduced downtime)
- Elimination of diatomaceous earth (reduction in procurement and disposal costs and in health risk to personnel)
- Reduction in manpower
- More consistent product quality (elimination of filter cake breakthroughs)
- Reduced prep time (no need to “build” a filter cake)
- Reduced downtime of clarification equipment

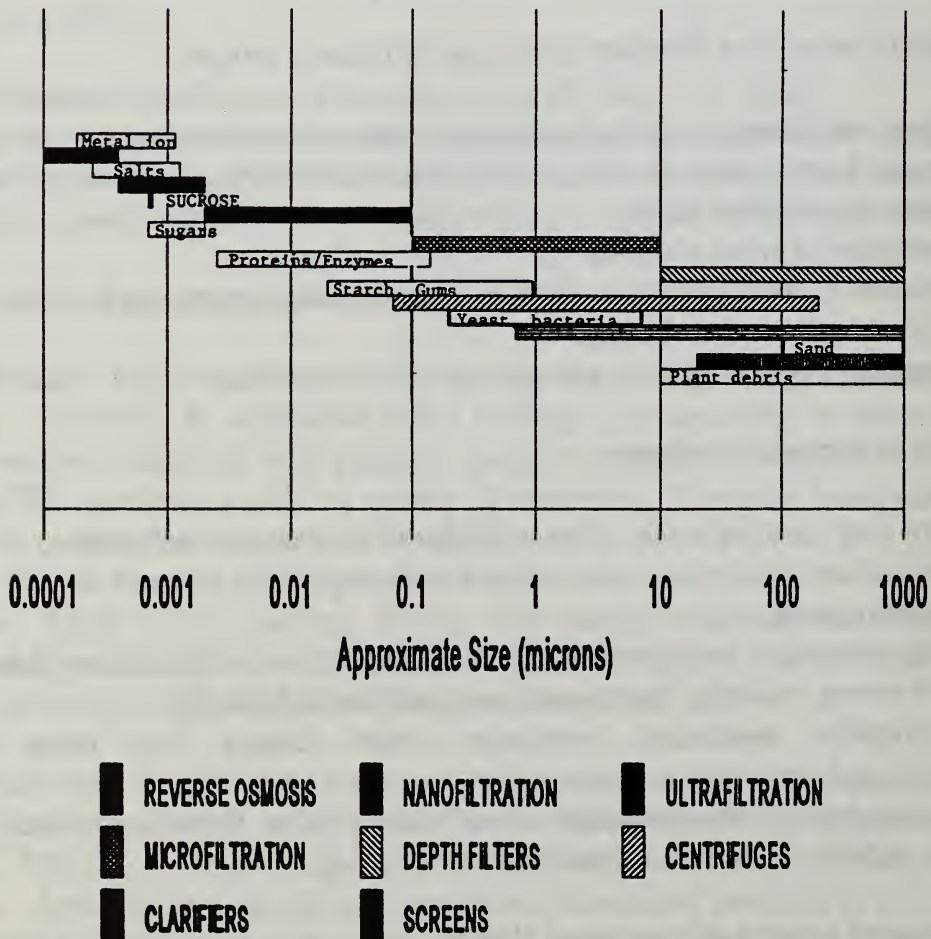


Figure 1. Common separation technologies and sizes of some species of interest.

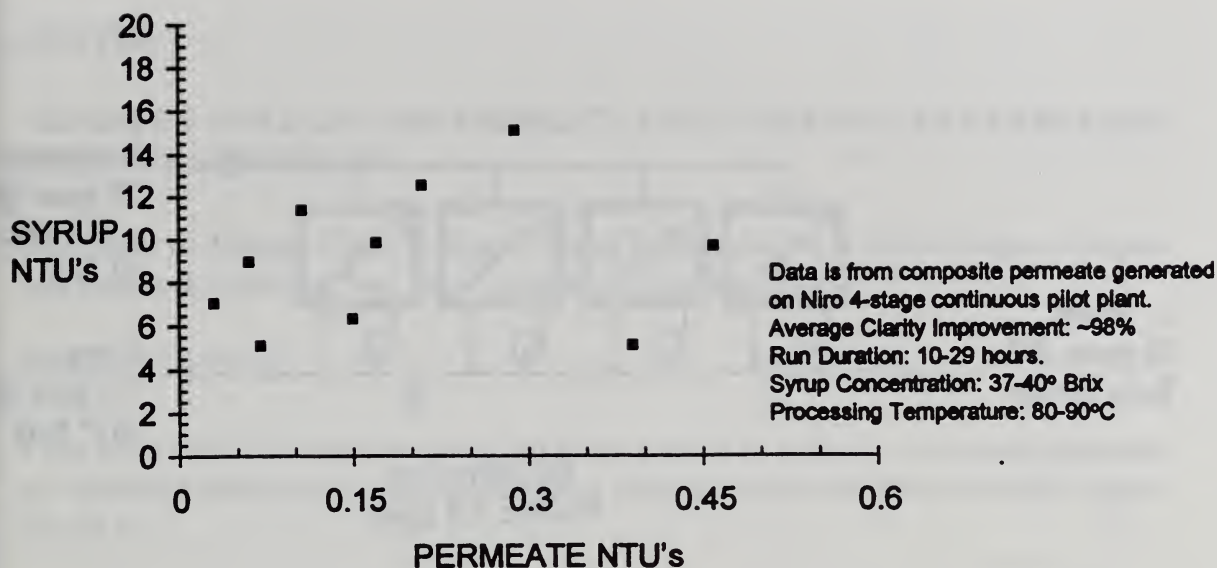


Figure 2. Purification of low purity refinery syrups with Membralox 500A ceramic membranes: Turbidity reduction on "Syrup #1".

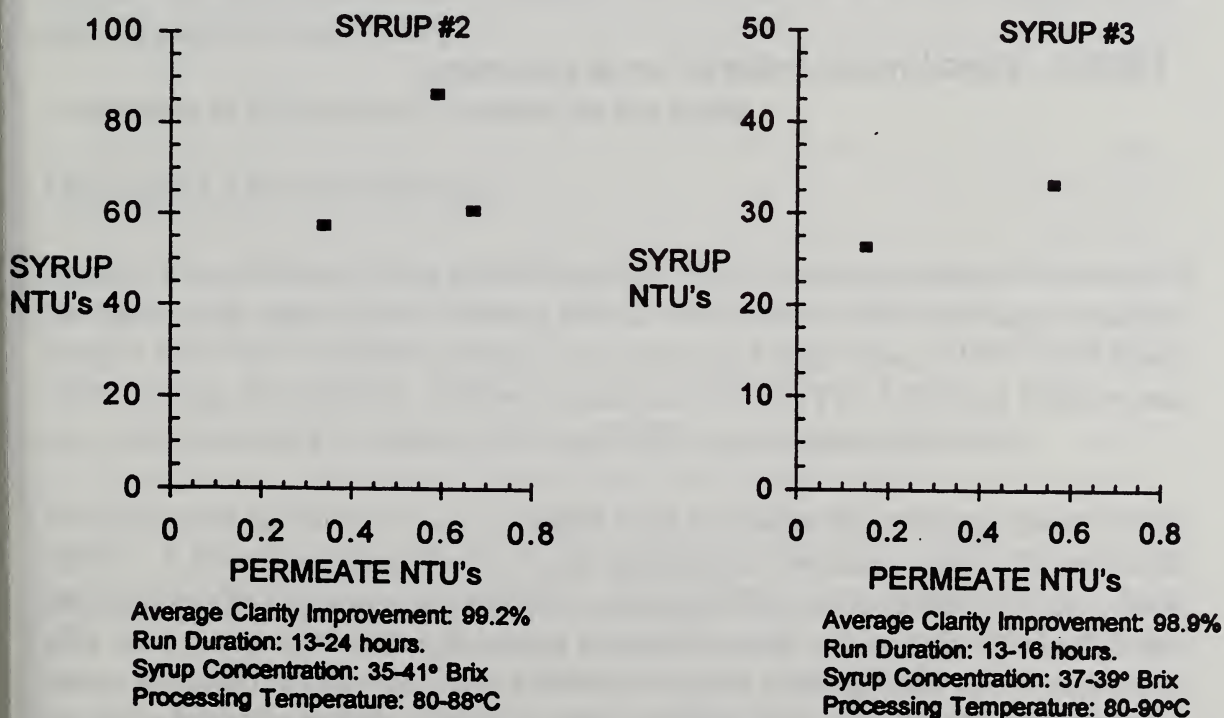


Figure 3. Purification of low purity refinery syrups with Membralox 500A ceramic membranes: Turbidity reduction on syrups "#2" and "#3".

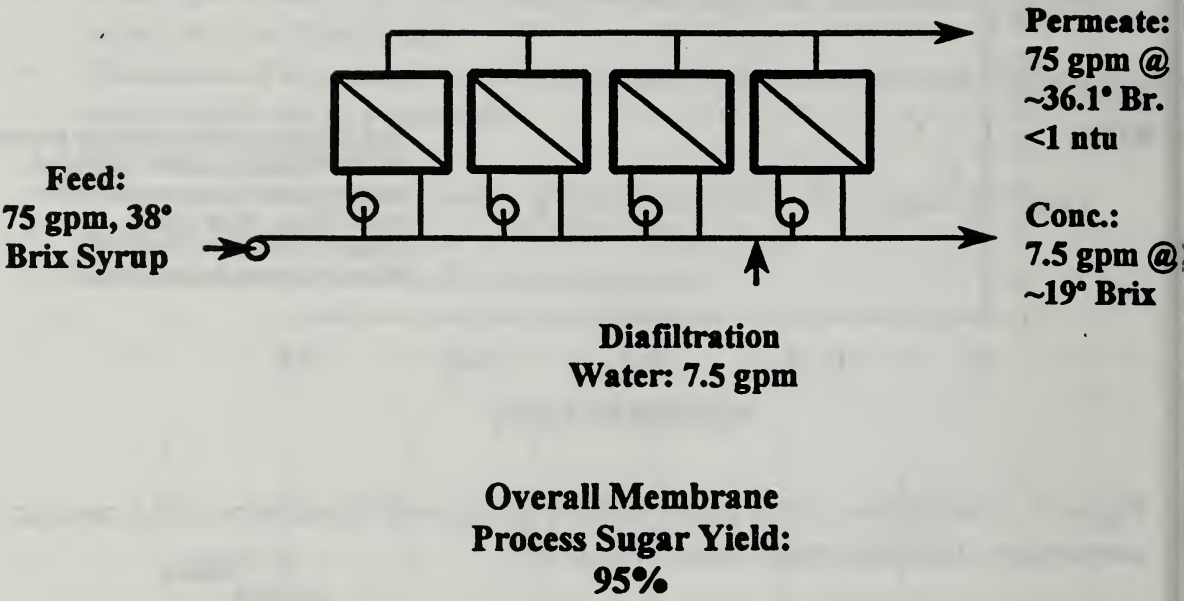


Figure 4. Typical system design for syrup processing.

POSTER

REFINERY STREAM AND PRODUCT ANALYSES BY NEAR INFRARED (NIR) SPECTROSCOPY

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INTRODUCTION

Near infra-red (NIR) spectroscopy has been applied to refinery process streams and to standard analyses of raw sugars and refined white and brown (soft) sugars. Goals are:

- (1) to increase availability of information on process streams that are seldom analyzed, e.g., remelt and low purity material,
- (2) to replace lengthy and labor-intensive methods for raw and refined sugar analyses, e.g., moisture, grain size.

A selection of NIR methods is shown on this poster.

METHODS AND MATERIALS

Spectra were obtained using an NIRSystems 6500 scanning spectrophotometer, in the visible-NIR range (400-2500 nm) and an NIRSystems 5000 Beverage Analyzer, range 1100-2500 nm (NIRSystems, Inc., Perstorp-Tecator Inc., 12101 Tech Road, Silver Spring, MD 20904). Software used was NSAS (Ver. 3.3) from NIRSystems Inc.; this was used for both the 6500 and 5000 (see below) instruments.

Samples were all diluted on a 1:1 weight basis to enable the pumping system to run easily. A flow-through cell of 0.5 mm pathlength was used, with reference scan made when the cell was clean and dry, and stored for use in sample analysis. Samples were pumped into the cell using a peristaltic pump at low rate. It was not necessary to wash the cell with water between samples, although this was done periodically to flush the system. Samples were not filtered or clarified, but poured into a beaker on a top-loading balance; an equal weight of water was added to the beaker and the sample stirred before being pumped into the NIR cell. It was possible to

run samples undiluted, but high viscosity samples required higher pump settings and often forced tubing connections apart.

Reference method lab analyses were run using standard laboratory methods: Brix by refractometer; pol (589 nm) after clarification with calcium hydroxide and aluminum chloride. Some lab analyses were run by refinery personnel; analyses additional to normal shift samples were run, on site, by S.P.R.I. personnel.

A second type of NIR spectrophotometer, Model 5000, called a "Beer Analyzer" was also used for liquid samples. On this instrument, two probes are fixed 1 mm (variable) cm apart, and a beaker of liquid sample is introduced so that the electrodes are immersed in the sample. This instrument analyses only liquid samples, and reads in the 1100 to 2500 nm (cannot read color), but is about 30% cheaper than a Model 6500.

RESULTS AND DISCUSSION

An increase in available data on low purity streams will be particularly helpful in controlling addition of water. For this reason, the Brix measurement is emphasized in this discussion.

Data collected for calibration sets for Brix, pol and purity are outlined in Table 1. Purity calibrations are shown, although in most cases it is better to calculate purity from the NIR measurements of pol and Brix. These sets of medium run-off or green syrups, of remelt syrups, and of final molasses, have all been calibrated in their individual sets, and also combined into a single set for a master, or universal, calibration.

Results of validation sets of data (additional samples run on NIR but not included in the calibrations) run against the three universal calibrations, for Brix, pol and purity are shown in Table 2.

Results of validation sets for each of the individual set, on individual calibrations, are shown in Table 3.

Comparison of data for pol and Brix (see Table 4) shows that, when individual calibrations are used, the standard errors of prediction (S.E.P.) are generally lower by some 10% than the S.E.P.'s of the data yielded by the universal calibration. The

small range of the individual data sets will lead to lowered error. Since the data will be obtained for the purpose of observing trends, rather than making precise single measurements, it appears that the universal calibrations are satisfactory for general use. The same universal calibration curves, for 26-82 Brix and 26-71 pol, have been tried at several plants: cane sugar refineries, sugarcane factories and sugarbeet factories, and have predicted satisfactory results in all places.

The value of the universal calibration for companies with groups of refineries and factories is obvious - each unit in the corporation will have data readily comparable to that of every other unit (Figures 1a, 1b, 1c).

Analysis of sugars

A single NIR scan of a raw sugar can provide results for component by two or more of several methods available. This facility is especially useful when methods are being evaluated. In the example shown, a calibration of raw sugar moisture by the traditional oven-drying method is shown alongside a calibration of data from the Omnimark infrared dryer (Figures 2a and 2b). In this way, results from two different methods can be obtained rapidly and simultaneously to satisfy specifications of different customers.

Analysis of soft sugars

Analysis of several parameters of soft sugars can be achieved in a single scan (a one-minute analysis), as shown below by calibration curves for moisture, solid Agtron color and ash. Light, medium and dark soft sugars are all combined in a single calibration for each component (Figures 3a, 3b, 3c). Results for the three analyses can be printed out simultaneously from a single NIR analysis.

Comparison of sample presentation methods on affination syrup

Refinery affination syrup samples (a set of 160) were run on an NIR in two different presentations. A 1:1 weight dilution of each sample was run in each case, on an NIRS6500. The two systems, with results on Brix analysis in affination syrup, were:

	NIRS6500, flow-through cell analyzer with fixed probes	Beverage analyzer
Correlation coeff.	0.99	0.95
Std. Error of calibr.	0.55	0.96

For ease and speed of operation, the Beverage Analyzer offers a slightly lower accuracy and precision than the flow through cell.

Effect of refinery A calibration on refinery B samples

Substrate: Affination syrup

Calibration: Universal calibration, low purity material, refinement, Brix measurement

Correlation: 0.985

Standard error of performance: 0.56

However: pol calibration had error >1.0

CONCLUSIONS

Calibrations can be transferred among plants, if laboratory (reference) methods are same. In refineries A and B, Brix measurement was made by same method; pol measurement was not.

Table 1. Samples in the universal calibrations.

SET	BRIX RANGE	POL RANGE	PURITY RANGE
Medium green, or run-off	74.1-81.9	39.1-50.5	47.7-63.7
Remelt	26.5-72.8	25.8-70.5	77.3-99.3
Final molasses	62.6-77.4	43.2-63.5	64.9-88.2
Overall ranges	26.5-81.9	25.8-70.5	47.7-99.3

Table 2. Calibrations with errors and validations with predicted errors for each set, using the universal calibrations.

Set	Calibration			Validation	
				r	Std. error of prediction (SEP)
Medium run-off	Brix	0.99	0.90	0.99	0.46
	pol	0.99	0.87	0.96	0.51
Remelt	Brix	0.99	0.90	0.99	1.23
	pol	0.99	0.87	0.97	0.61
Final molasses	Brix	0.99	0.90	0.95	0.57
	pol	0.99	0.87	0.93	0.88

Table 3. Calibrations and predicted errors for each set with its own validation set, using individual set calibrations (on small data sets).

		Calibration		Validation	
		r	S.E.C.	r	S.E.P.
Medium run-off	Brix	0.93	0.63	0.99	0.42
	pol	0.90	0.52	0.98	0.41
	purity	0.97	0.68	0.87	0.59
Remelt	Brix	0.99	0.61	0.98	0.63
	pol	0.99	0.77	0.98	0.53
	purity	0.85	0.78	0.80	1.14
Final molasses	Brix	0.97	0.49	0.95	0.58
	pol	0.98	0.58	0.96	0.62
	purity	0.89	0.81	0.89	0.76

Table 4. Comparison of standard error of prediction using universal calibration and using individual calibrations.

		Standard error of prediction	
Set		Universal calibration	Individual calibration
Medium run-off	Brix	0.46	0.42
	pol	0.51	0.41
Remelt	Brix	1.23	0.63
	pol	0.61	0.53
Final molasses	Brix	0.57	0.58
	pol	0.88	0.62

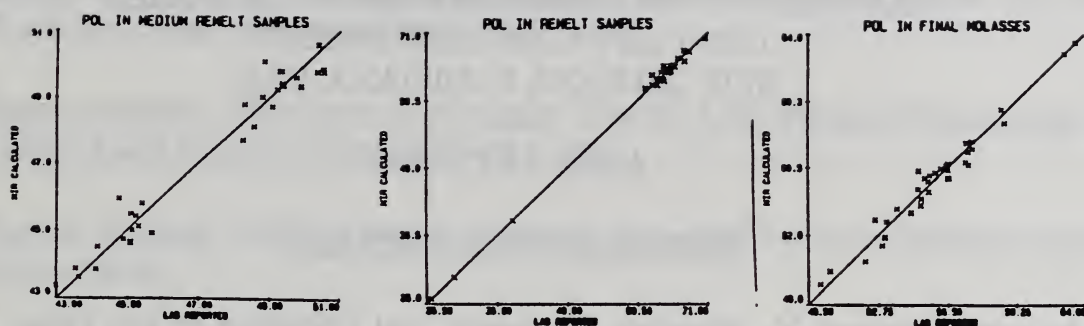


Figure 1. NIR calibrations of low purity streams.

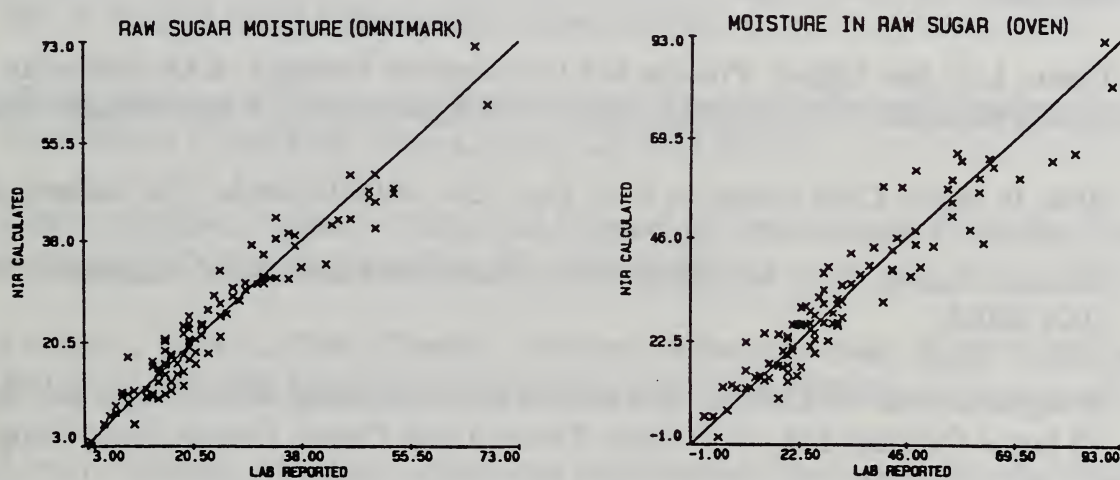


Figure 2. NIR comparison of two analyses for moisture in raw sugars.

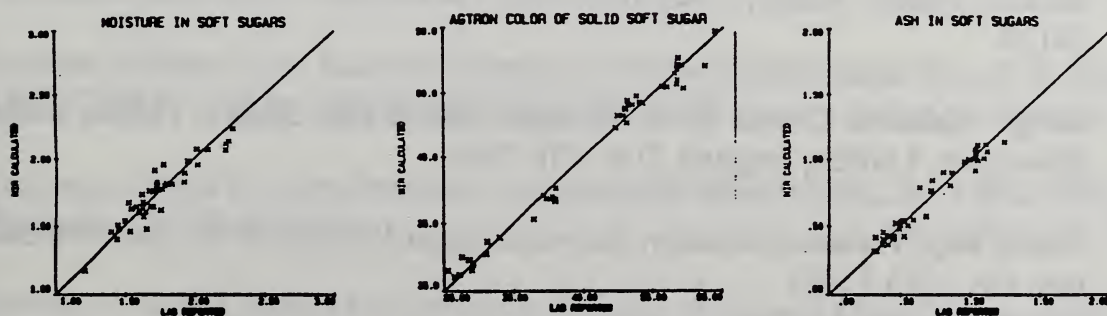


Figure 3. NIR analyses of soft sugars.

CONFERENCE ON SUGAR PROCESSING RESEARCH
OMNI-ROYAL ORLEANS HOTEL
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